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37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date below:

June 21, 2004
Date


Steven L. Highlander

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Dennis E. HALLAHAN *et al.*

Serial No.: 08/540,343

Filed: October 6, 1995

For: METHODS AND COMPOSITIONS FOR
VIRAL ENHANCEMENT OF CELL
KILLING

Group Art Unit: 1632

Examiner: S. Priebe

Atty. Dkt. No.: ARCD:194/SLH

SECOND SUPPLEMENTAL INVENTORS' DECLARATION UNDER 37 C.F.R. §1.131

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

We, the undersigned, do declare that:

1. We are citizens of the United States and named inventors on the above-captioned application.

2. One or more of the present inventors, or person(s) acting under their direction and control, were actively engaged in activities in this country relating to a reduction to practice of

the claimed invention from prior to June 23, 1994 to the filing date of the above-captioned application.

3. Attached to this declaration are redacted notebooks evidencing activity from about July 31, 1994 to October 6, 1995. Previously submitted materials showing work conducted prior to June 23, 1994.

4. Applicants have been unable to identify notebooks containing experimental results from the period of June 23, 1994 to July 31, 1994. However, during this time frame, the present inventors were discussing the present invention in the context of planning experiments, ordering of materials, or preparing to conduct experiments on at least a weekly (if not daily) basis in the period of time between June 23, 1994 and July 31, 1994. Thus, taken together, the evidence of record reflects continuous activity from before June 23, 1994 to applicants' filing date of October 6, 1995.

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

May 24, 2004
Date


Dr. Ralph Weichselbaum

Date

Dr. Dennis Hallahan

Date

Dr. Gregory Sibley

Date

Dr. Donald Kufe

Date

Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date

5/26/04
Date

Dr. Ralph Weichselbaum

Dennis Hallahan
Dr. Dennis Hallahan

Date

Dr. Gregory Sibley

Date

Dr. Donald Kufe

Date

Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date

Dr. Ralph Weichselbaum

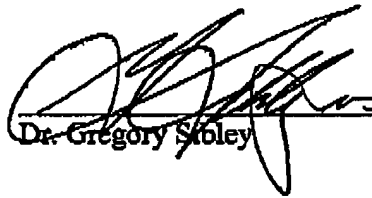
Date

Dr. Dennis Hallahan

Date

6/18/04

Dr. Gregory Stoley



Date

Dr. Donald Kufe

Date

Dr. Bernard Roizman

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Date

Dr. Ralph Weichselbaum

Date

Dr. Dennis Hallahan

Date

Dr. Gregory Sibley

Date

5/25/04

Dr. Donald Kufe

Date

Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date

Dr. Ralph Weichselbaum

Date

Dr. Dennis Hallahan

Date

Dr. Gregory Sibley

Date

Dr. Donald Kufe

June 10 2004
Date



Dr. Bernard Roizman

Current medium for propagation: Eagle's MEM with non-essential amino acids, sodium pyruvate, 1 mM and Earle's BSS, 90%; fetal bovine serum, 10%.

This is one of a number of cell lines derived from malignant gliomas (See also ATCC HTB 16, 17) by J. Ponten and associates from 1966-69 (Acta Pathol. Microbiol. Scand. 74: 465-486, 1968; Hum. Hered. 21: 238, 1971). Cultures were established as explants on grid-supported lens paper or gelatin foam with Eagle's minimum essential medium and 10% bovine calf serum as the culture fluid. Trypsinization of the outgrowth or cells attached to the vessel floor with subsequent transfer to standard vessels in growth medium permitted cell line development. A culture at passage 108 was deposited by J. Ponten in July, 1973. Mycoplasma contamination was eliminated in September, 1975.

HUMAN TUMOR CELL BANK — HTB

ATCC HTB 14 (continued)

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-44; Sex-Female; Race-Caucasian; Blood Type-A⁺.

Grown as: Monolayer; transferred 1:5 weekly.

Morphology: Epithelial-like.

In Vitro Cytopathology: (P120) Consistent with glioblastoma.

Nude mouse: Produces malignant tumor consistent with glioblastoma.

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 122.

Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.

Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	2	3	1	15	21	7	1
Chromosomes:	40	41	42	43	44	45	47

The stemline chromosome number is hypodiploid, the 2S component occurring at 5.4%. Nine markers [t(1q;23), t(1p22q), t(6p?11q-), t(6q?7p), t(7q;?), del(12q), t(20;1p;9q), t(28p;?), and M1] were common to most S metaphases. Neither HSR's nor DM's were detected. The line was originated from a female patient. However, all S metaphases were monosomic for the X chromosome.

Viability: 93%.

Culture Medium: Eagle's minimum essential medium with non-essential amino acids, sodium pyruvate and Earle's BSS, 85%; fetal bovine serum, 15%; antibiotic-free.

Isoenzymes: Me-2, 1; PGM₁, 1; PGM₂, 2; ES D, 1; AK1, 1; GLO-1, 1; G6PD, B.

Phenotype Frequency Product: 0.0017.

Sterility: Tests for mycoplasma, bacteria and fungi were negative.

Species: Confirmed as human by isoenzyme analysis.

Note: This material is available under the conditions that you will not use it for commercial purposes or distribute it to third parties. Please see pages xv and xvi for the form required.

Price Code: J

ATCC HTB 15

U-118 MG

(Glioblastoma, human)

Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%.

This line is one of a series derived by J. Ponten and associates as discussed under ATCC HTB 14, 16 and 17 (Acta Pathol. Microbiol. Scand. 74: 465-486, 1968). The source tumor was described as a grade III astrocytoma-glioblastoma with one area resembling an ependymoblastoma. Cytoplasmic granulation was striking and astroblasts with neurofibrils were observed. Spongioblasts were abundant in culture and were not affected by frequent subcultivation.

A culture at passage 416 was provided originally by J. Ponten. Progeny transferred to the ATCC in 1982 were found to be contaminated with mycoplasma. The infection was cured in 1987 by treatment with BM cycline over a 6-week culture period.

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-50; Sex-Male; Race-Caucasian; Blood Type-A⁺.

Grown As: Monolayer; transferred 1:3.5 weekly.

Morphology: Mixed.

Karyology: Hypopentaploid to hyperpentaploid with abnormalities including breaks (P419).

In Vitro Cytopathology: All spindle giant cells, malignant.

Nude mouse: Yields pleomorphic malignant tumor consistent with glioblastoma multiforme invading muscle.

HLA Cell Line Phenotype: AW 24, 28; B12, W47 (Pollack, et al.).

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 443.

Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.

Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	1	1	3	1	2	4	1	4	1	3	4	2	3	3	5	1	2	2	2	1	1	2	1
Chromosomes:	71	99	102	103	104	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	123	125

CELL REPOSITORY LINES — CRL

ATCC CRL 1690

T98G (Glioblastoma, human)

†Passage Frozen: 427. Current medium for propagation: Eagle's MEM with non-essential amino acids, 1.0 mM sodium pyruvate and Earle's BSS, 90%; fetal bovine serum, 10%. Additional Information: This line was derived by L. Hayflick from a glioblastoma multiforma tumor from a 61-year-old male Caucasian. T98G has an indefinite lifespan and is anchorage-independent, but can enter a viable G1-arrested state when crowded or deprived of serum. These cells should be useful for studies on the mechanisms for cessation of proliferation in quiescent cells, and for studies requiring cells synchronized in G1 phase. This is a hyperpentaploid human cell line. The modal chromosome number should be around 128 to 132. The rate of cells with higher ploidies was 1.39%. Fourteen to 16 marker chromosomes were common to most cells. Reference: J. Cell. Physiol. 99: 43-54, 1979. Submitted by: G.H. Stein, University of Colorado, Boulder, CO. Price Code: J

ATCC CRL 1691

C7 (Mouse hybridoma, anti LDL receptors)

Complete description appears in the Hybridoma Section of the Catalogue (pp. 333-349).

ATCC CRL 1692

HISM (Smooth muscle, jejunum, human)

†Passage Frozen: 12; PDL 16. Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%. Additional Information: This cell line was derived from the *muscularis propria* of the jejunum of a normal 35-year-old female patient. It synthesizes collagen and contains actin stress fibers. It contracts in response to the C-terminal octapeptide of cholecystokinin. Reference: Proc. Soc. Exp. Biol. Med. 176: 503-507, 1984. Submitted by: M.F. Graham and R.F. Diegelmann, Medical College of Virginia, Richmond, VA. Price Code: J

ATCC CRL 1693

NFS-5 C-1 (Pre-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-2SM ecotropic murine leukemia virus. The cells bear a pattern of markers consistent with identification as large pre-B lymphoblasts (i.e., Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). The cells spontaneously produce both ecotropic and mink cell focus-forming viruses. Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1694

NFS-70 C-10 (Pro-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-NS-7 ecotropic murine leukemia virus. The cells bear a pattern of markers suggestive of pro-B lymphoblasts (i.e., Mac-1+, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). The cells appear to be of a very early stage of commitment to B-cell differentiation. Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1695

NFS-25 C-3 (Pre-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a spontaneously arising lymphoma in an NFS.C58v-1 mouse. The cells bear a pattern of markers consistent with identification as pre-B lymphoblasts (i.e., Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson, and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1696

McCoy (Mouse)

†Passage Frozen: Unknown. Current medium for propagation: Eagle's MEM with non-essential amino acids and Earle's BSS, 90%; fetal bovine serum, 10%. Additional Information: Little descriptive information about the origin of the McCoy cells appears in the literature. They were first mentioned by Pomerat, et al. (Z. Zellforsch. 47: 158-174, 1957). The cells have originated from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis. It was shown that McCoy cells (designated McCoy A) were indeed human cells. However, another subline was shown in fact, of mouse origin and possessed marker chromosomes characteristic of strain L mouse. It was assumed to be human, but which actually are mouse cells, have been disseminated from laboratory sources. Initial interest in McCoy cells followed the demonstration by Gordon and Quan (Proc. Natl. Acad. Sci. 65: 123-129, 1968) and Gordon, et al. (Appl. Microbiol. 23: 123-129, 1972) that ionizing radiation induced mutagenesis of McCoy cells to infection by chlamydia strains. A culture of the so-called McCoy-1 strain was deposited with the American Type Culture Collection, Cell Culture Department, Atlanta, GA in March, 1984. The cells have been used to propagate laboratory strains of chlamydia. The cell line has been satisfactory for chlamydia growth for at least 10 years. Price Code: J

(receptors)

See (pp. 333-349).

median of

titrate down vials, time ($< 24^{\circ}$), ↓ serum

		10^8 PFU	10^3	10^6	
HSV-R (425-TNF)		$5 \times 10^8 / \text{ml}$	(particular)		MOI = 5 $\therefore 10^8$
HSV-F \emptyset		$10^8 / \text{ml}$			

Cells (TNF)

SQ 208

HS 638 (glione)

- 1) SQ more sensitive
- 2) PFU & time depend cytotoxic in each
- 3) 0.1 PFU more than enough
- 4) 24° may be too long for induction since virus cytolytic

Titrate down

- 1) PFU of .02 gave 1000 pg/ml / .002 240-400 pg/ml
- 2) no induction

Survival

- 1) both \emptyset & +TNF cytolytic
- 2) both enhanced XR kill even at 1 day

"

\emptyset

- cell density dependent cytotoxicity
- cytotoxicity transferred in medium

PFU .001 - little cytotoxic but (+) enhance of XR kill

Program

low PFU \rightarrow hardly infected

copy pg 83

Decanted CM

confluent x 4 days unfed

1/200 MSV \pm XR (36g) 6 hrs later

18hr decant & feed

80% or 20% of each onto confluent cells

all 1/200 MSV

Ø fed

Ø fed \rightarrow subculture

36g fed

36g fed \rightarrow subculture

20% Ø CM

20% Ø CM \rightarrow subculture 24°

20% 36g

20% 36g \rightarrow subculture 24°

80% Ø

80% Ø \rightarrow sub 24°

80% 36g

80% 36g \rightarrow sub 24°

24h CM Ø \rightarrow ? sub 24° ? \pm XR

24h CM 36g \rightarrow ? sub 24° ? \pm XR

48h CM Ø

48h CM 36g

$$\begin{array}{r} 0 \\ 40 \quad 122 \quad 200 \\ \hline 100 \quad 500 \quad 500 \end{array}$$

$$\begin{array}{r} 0 \\ 40 \quad 152 \quad -200 \\ \hline 500 \quad 10 \quad 10^4 \end{array}$$

$$\begin{array}{r} 0 \\ 79 \quad 100 \quad 236 \\ \hline 200 \quad 100 \quad 500 \end{array}$$

$$\begin{array}{r} 0 \\ 100 \quad 250 \quad 71000 \\ \hline 200 \quad 500 \quad 10 \quad 10 \end{array}$$

$$\begin{array}{r} \text{Confluent} \\ 51 \quad 43 \quad 162 \\ \hline 200 \quad 100 \quad 500 \end{array}$$

$$\begin{array}{r} \text{Confluent} \\ 253 \quad 21000 \quad 500 \\ \hline 500 \quad 10^4 \quad 10^3 \end{array}$$

$$\begin{array}{r} 0/200 \quad 0/10^3 \quad 0/500 \\ 0/200 \quad 0/10^3 \quad 0/500 \end{array}$$

cell gets affected?

? toxin or
depletion or
some or
surface (over trigger
to confluent)

1) ? does cell density effect PE 2) ? is a new protein made after treatment

$$\begin{array}{ccc}
 & \text{PE} & \\
 40\% & \frac{300, 500, 10^3, 2 \times 10^3, 5 \times 10^3, 3 \times 10^4}{\downarrow} & \xrightarrow{\quad} \frac{3 \text{ Gy}}{\text{"}} \\
 4\% & \text{"} & \text{"}
 \end{array}$$

} on dishes for 1 hr
in 1 ml medium

② SQ2013 10^5 in 7-75 (2)

③ SQ2013 5×10^5 on p100 - 4 dishes \rightarrow 35g-methionine

$$\begin{array}{ccccccccc}
 & & \text{PE} & & & & 3 \text{ Gy} & & \\
 40 & \frac{30}{300} & \frac{30}{500} & \frac{200}{2000} & \frac{25}{5000} & \frac{15}{10^4} & \frac{20}{300} & \frac{20}{500} & \frac{30}{1000} & \frac{10}{5000} & \frac{25}{10^4} \\
 & & \frac{190}{1000} & & & & & \frac{100}{2000} & & & \\
 4 & \frac{30}{300} & \frac{50}{500} & \frac{780}{5000} & \frac{20}{10^4} & & \frac{10}{300} & \frac{25}{500} & \frac{0}{2000} & \frac{40}{5000} & \frac{40}{10^4} \\
 & & \frac{100}{1000} & \frac{120}{2000} & & & & & & &
 \end{array}$$

① What is the relative infectivity of HSV 3616 in SQ-20B compared to Vero cells?

② What level of ~~some~~ viral inoculum can nude mice tolerate?

③ At what PFU is TNF production maximized?

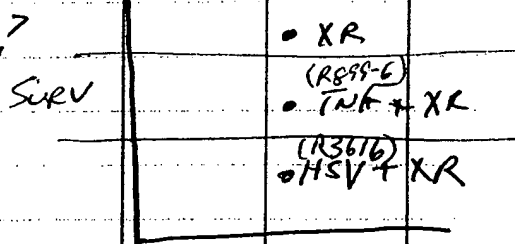
At what time point is TNF production maximized?

Compare wt HSV I to HSV 3616 to HSV 899-6

Does RT affect TNF production?

④ How does RT affect cytotoxicity?

where does HSV alone fall?



⑤ Does supernatant sensitize? How about supernatant to Vero cells?

Does supernatant retain infectivity or toxicity?

⑥ Is TNF produced in viral infection in RT?

⑦



	1	2	3	4	5	6	7	8	9	
1	Any reason to keep working in R899-6?									
2										
3										
4										
5										
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EFFICIENCY LINE® 22-206



[illegible]

[illegible]

Doubling Times

	1	2	3	4	5	6	7	8	9	
1	SQ-10B - 10 ⁵ cells plated in T-25. On d 4 = 50% confluent									
2										
3										
4										
5										
6										
7										
8										
9	SK-N-SH - 10 ⁵ cells plated in T-25. On d 4 = 80-90% confluent									
10										
11										
12										
13										
14										
15										
16										
17	Vero cells -									
18										
19										
20										
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MEDIUM 199/1% calf serum(pH7.6-7.9)

Application: virus propagation and titration.

Preparation: 1X 199v

room T⁰ sterile glass distilled water.....440mls
10X 199 Hank's BSS w/o L-glutamine(Hazleton)..... 50mls
1000X Pn/Strept.....0.5mls
heat inactivated bovine calf serum(Colorado Serum).. 5mls
100X L-glutamine(Sigma)..... 5mls
7.5%(w/v)NaHCO₃(Sigma).....9.35mls

(1) NaHCO₃ must be added last.

(2) NaHCO₃ concentration must be 1.4g/l in the 1X formulation. The color of the 1X medium after addition of NaHCO₃ should be cherry red. If necessary, add 6N NaOH dropwise to achieve the desired color. DO NOT increase amount of NaHCO₃.

(3) Store at 4°C; shelf life 4-6 weeks.

Preparation: 1X 199v

1X 199v.....500mls
pooled human gamma globulin.....0.5mls

To 500mls 1X 199v aseptically add 0.5mls(0.1%) pooled human immunoglobulin.

Shelf life: suggest preparing fresh.

Gibco
Medium 199 = Cat No 11181-021
10 X
c Hanks salts
c L-glut
5 NaHCO₃

GIEMSA STAIN

Application: , Solution used to stain viable cells; specifically used in cell culture for virus titration assay.

Source: Sigma

Preparation: 10X GIEMSA

Time Element: 16 day period

giemsa powder.....5 grams

glycerol.....500mls

methanol.....500mls

- Day1 (1) Put giemsa and glycerol into a flask which has a layer of glass beads .
(2) Place flask on a shaker at 37°C; shake overnight.
- Day2 (3) Remove flask from 37°C and add methanol.
(4) Mix(with stir bar)at room temperature .
(5) Place in dark for 2 weeks(This can be achieved by completely wrapping flask with foil.) at room temperature.
- Day16 (6) Filter solution with Whatman #1 paper.
(7) Store at room temperature. No known expiration.

Strengths:

Stock: 10X

Working: 1X (Prepare day of use by diluting 10X stock 1:10 with glass distilled water;
eg: 1ml 10X + 9mls water).

Use 7.5^{ml} (10 x stock) ~~in 50 ml wt~~ + 42.5 ml dH₂O

SQ20B: 3616 vs 899-6, 10^{-1} vs 10^{-3} MOI

SIBLEY

Purpose

- Quantify cell Killing \bar{c} R3616 & R899-6 vs. no virus following 5 Gy RT.
- Does the TNF construct in R899-6 lead to additional cell kill over R3616?
- Quantify TNF production in serum and pellet after RT & infection.
- Compare cell killing ~~with~~ and TNF production with MOI of 10^{-1} , 10^{-3} .

Design

- Fixed Variables - Cell type: SQ-20B
RT dose: 5 Gy single fraction
Interval infect \rightarrow RT: 16 hrs
Interval RT \rightarrow subculture: 4 hrs
- Study Variables - Virus: None, R3616, R899-6
MOI: 10^{-1} PFU/cell, 10^{-3} PFU/cell

Methods -

① Plate 10^6 SQ-208 cells / 60 mm dish.

Volume/dish = 3-4 ml 20% FCS in DME + P/S

Incubate to 95% confluency (overnight)

Label plates: 10^{-1} MOI R3616 10^{-3} MOI R3616

(duplicate) 10^{-1} MOI R3616 10^{-3} MOI R3616

10^{-1} MOI R899-6 10^{-3} MOI R899-6

10^{-1} MOI R899-6 10^{-3} MOI R899-6

Ø virus

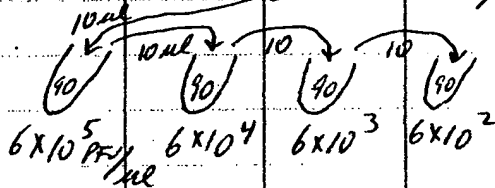
Ø virus

∴ need 10 plates +
1 extra to count cells

② Count a plate using hemocytometer = 1.12×10^6 cell.

③ Make dilution of viruses:

$$R3616 = 6 \times 10^9 \text{ PFU/ml} = 6 \times 10^6 \text{ PFU/}\mu\text{l}$$



R899-6 = $6 \times 10^9 \text{ PFU/ml}$ also, ∴ make same dilution.

④ Change media on plates to 2% FCS in DME, 2 ml/pl.

⑤ Add virus (12 noon)

$$\text{For } 10^{-1} \text{ MOI need } \frac{1.12 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/}\mu\text{l}} = 1.87 \mu\text{l of } 6 \times 10^4 \text{ dilution.}$$

$$\text{For } 10^{-3} \text{ MOI need } \frac{1.12 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU/}\mu\text{l}} = 1.87 \mu\text{l of } 6 \times 10^2 \text{ dilution.}$$

(optimally want vol of viral inoculum ~ 2-3 μl)

- 1
2 Methods (cont.) -
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31
- ⑥ Incubate 2 hrs, then add 2 more mls of 2% FCS (2^{pm})
 - ⑦ Next am (11^{am}) 5 Gy RT. (Garry dishes in sealed
Tupperware container). Use Cu-At filter, 250 KV, 26 mAmp.
50 cm plate-diaphragm distance. Time = 4'48"
 - ⑧ Label eppendorf tubes for TNF assay samples =
cm (conditioned media) & pellet for each dish.
 - ⑨ Label dilution tubes for each plate = 10^6 , 10^5 , 10^4 , 10^3
Add 9 ml of media to each tube (except 10^6 tubes)
 - ⑩ Label 100 mm plates. Need 3 plates per original
Plate for plating dilutions = 10^4 , 5×10^3 , 10^3 .
Add 10 cc of 20% FCS media to each plate
 - ⑪ Subculture dishes 4 hrs p RT (3^{pm}). Do 4 at
a time. Count 1 of each duplicate dish in
cytometer to resuspend to 10^6 . (counts were
 $\sim 37 \times 10^4$ cells/ml $\times 5$ mls = resuspend in 1.85 mls)
(Used 1 ml Versene + 1 ml Trypsin + 3 ml media = 5 ml
for subculturing). Put samples on ice.
 - ⑫ Make dilutions of each sample $10^6 \rightarrow 10^5 \rightarrow 10^4 \rightarrow 10^3$
 - ⑬ Plate dilutions in 100 mm plates @ 10^4 , 5×10^3 & 10^3
 - ⑭ Save 1 ml of supernatant after centrifuging as "cm"
(conditioned media). Save 1 ml of 10^6 dilution as "p"
(pellet) in eppendorf tubes, for TNF assay. Put
in freezer.
 - ⑮ Incubate plates for ~ 1 week
 - ⑯ TNF assay - see next sheet

TNF-ELISA RESULTS

	1	2	3	4	5	6	7	8	9
1	<u>R3616</u>		<u>[TNF] pg/ml</u>					<u>[TNF] pg/ml</u>	
2	10^{-3}	CM	0			10^{-3}	CM	0	
3	10^{-3}	CM	0			10^{-1}	CM	0	
4	10^{-3}	P	0			10^{-1}	P	0	
5	10^{-3}	P	0			10^{-1}	P	0	
6									
7	<u>R899-6</u>		<u>[TNF] pg/ml</u>					<u>[TNF] pg/ml</u>	
8	10^{-3}	CM	0			10^{-1}	CM	25	32.7
9	10^{-3}	CM	0			10^{-1}	CM	25	32.3
10	10^{-3}	P	0			10^{-1}	P	44	51.5
11	10^{-3}	P	0			10^{-1}	P	170	56.6
12									
13	<u>Ø virus</u>		<u>[TNF] pg/ml</u>						
14		CM	0						
15		CM	0						
16		P	0						
17		P	0						
18									
19									
20	CM = conditioned media								
21	P = pellet (resuspended @ 10^6 cells/ml)								
22	$10^{-3}, 10^{-1}$ = Viral MOI								
23									
24									
25									
26									
27									
28									
29									
30									
31									

ELISA

Standards

0 med.	1	9	17
15.7	2	10	
33.1	3	11	18
67.5	4	12	
125	5	13	19
250	6	14	
500	7	15	20
1000	8	16	

Put TNF in 1ml distilled H₂O

1. dilute standards in 0.5ml R05 buffer (1:2)
2. Add standards (+ media only) 200ul to duplicate wells
3. Add 200ul of sample to each well, cover
shake 1 hr.
4. Wash 3X by aspirating liquid, adding ~200ul of wash buffer

5. Add 200ul of conjugate A
shake ~30 min.

6. Wash again
(make up A+B)

7. A 200ul A+B

1. 3616 10^{-3} cm 17. Φ v. r. s. cm

2. 3616 10^{-3} P 18. ~~ammonia~~

3. 3616 10^{-1} cm 19. Φ v. r. s. P

4. 3616 10^{-1} P 20. ~~ammonia~~

5. 899-6 10^{-3} cm

6. 899-6 10^{-3} P

7. 899-6 10^{-1} cm

8. 899-6 10^{-1} P

9. 899-6 10^{-3} cm

10. 899-6 10^{-3} P

11. 899-6 10^{-1} cm

12. 899-6 10^{-1} P

13. 899-6 10^{-3} cm

14. 899-6 10^{-3} P

RESULTS (cont.)

Colony counts

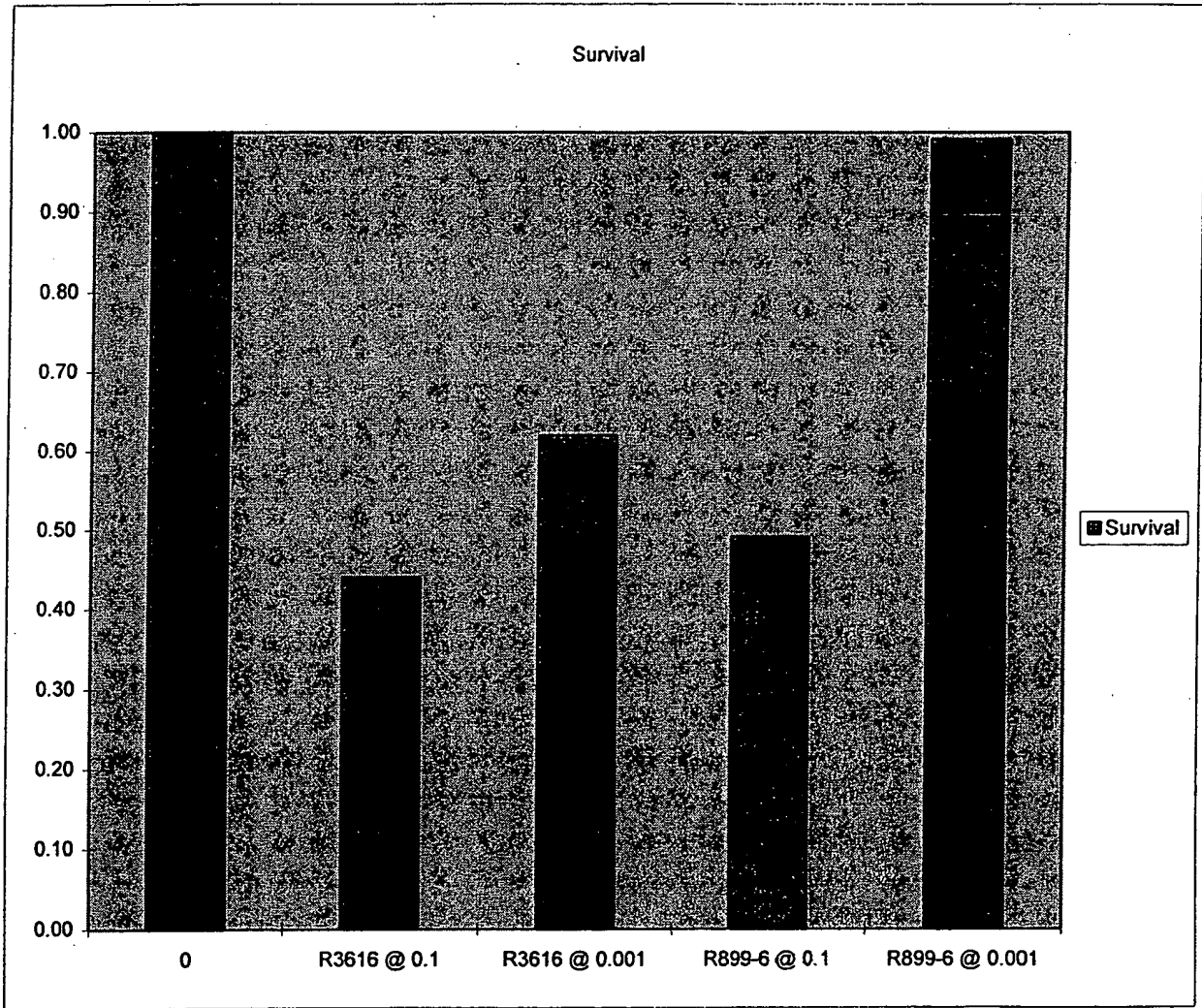
ovipos	virus	MOI	# cells plated	count		* Approx Survival	Relat. N. Efficiency
	Ø	0	10^3	144	> 176	35%	
	"	"	"	208			
R3616	"	10^{-1}	10^3	66	> 77.5	16%	
"	"	"	"	89			
"	"	10^{-3}	"	89	> 109	22%	
"	"	"	"	129			
R899-6	"	10^{-1}	10^3	105	> 86.5	17%	
"	"	"	"	68			
"	"	10^{-3}	"	176	> 174.5	35%	
"	"	"	"	173			
"	"	"	5×10^3	700			

* (Note: No control plated, i.e. Ø virus - Ø RT, ∴ can't determine plating efficiency)
 If assume P.E. of 50%

Results - Counts SQ20B

RT	Virus	# Plated	Ave. Coun	P.E.	Survival
5	0	1000	176	0.176	1.00
5	R3616 @ 0.1	1000	77.5		0.44
5	R3616 @ 0.001	1000	109		0.62
5	R899-6 @ 0.1	1000	86.5		0.49
5	R899-6 @ 0.001	1000	174.5		0.99
Virus	Survival				
0	1.00				
R3616 @	0.44				
R3616 @	0.62				
R899-6 @	0.49				
R899-6 @	0.99				

SQ



	1	2	3	4	5	6	7	8	9	
1	<u>Findings</u>									
2	1) In SQ-208:									
3	a. No detectable TNF production @ MOI 10^{-3} = R899-6 (+5 Gy)									
4	b. TNF detectable at MOI 10^{-1} (R899-6 +5Gy) with 55 pg/ml in pellet sample & 32 pg/ml in supernatant sample.									
5	(pellet sample = 10^6 cells/ml)									
6										
7	c. Despite TNF production, R899-6 did not produce more cell killing than R3616 (@ MOI 10^{-1} + 5 Gy)									
8										
9	d. Both viruses caused increased cell killing at an MOI of 10^{-1}									
10										
11	e. R3616 appeared to give more effective cell killing than R899-6 or Ø virus at MOI of 10^{-3} .									
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										



The University of Chicago Departmental Purchase Order

Purchase Order Number
Z 849078
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.
Not to be used for purchase of travel, hazardous or radioactive materials,
controlled substances, vehicle rental or other restricted items.

Vendor Name American Type Culture Collection
12301 Parklawn Dr.
Rockville State MD Zip Code 20852
Payment Terms _____ Delivery charge? ☐ Yes ☐ No
Telephone No. (800) 638-6597 FAX No. _____

THE UNIVERSITY OF CHICAGO Dept. Code: 1050
Radiation and Cellular Oncology
Department Michael Beckett Room G-03
Address 5830 S. Ellis Ave.
City Chicago State IL Zip Code 60637

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; second copy should be sent to vendor; third copy should be sent to the Purchasing Department.
4. Vendor and Ship to must have complete addresses.
5. Department Code MUST be filled in with department code number from listing already furnished.
6. Only ONE account code is allowed per order.

Order placed by phone? ☐ No ☒ Yes
Authorized Signature Mahmud Ghazvi Payroll No. _____
Print Name _____ Ext. 2-
Account Code 5-25754-5400 Date 8/8/94
Order placed with (name) Alonzo Date _____
Order placed by (name) Michael Beckett Phone # 248333

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	Flask	ATCC HTB 14	75.00	75.00
		Lab Fee	35.00	35.00
		Pkg. Fee	5.00	5.00
		Fed. Express	9.75	9.75
		Acct. # 2168		
DEPARTMENT COPY			ORDER TOTAL	120.75

NOT VALID IF TOTAL EXCEEDS \$500.00
BACK ORDERS ARE NOT ALLOWED

MOLECULAR DEVICES

Molecular Dynamics Macromolecular Spectrometer

PLATE #: 1

ASSAY: _____

DATE: _____

OPERATOR: SIBLEY O.D. LIMIT: 2.000

TIME: _____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

AUTO MIX: 33%

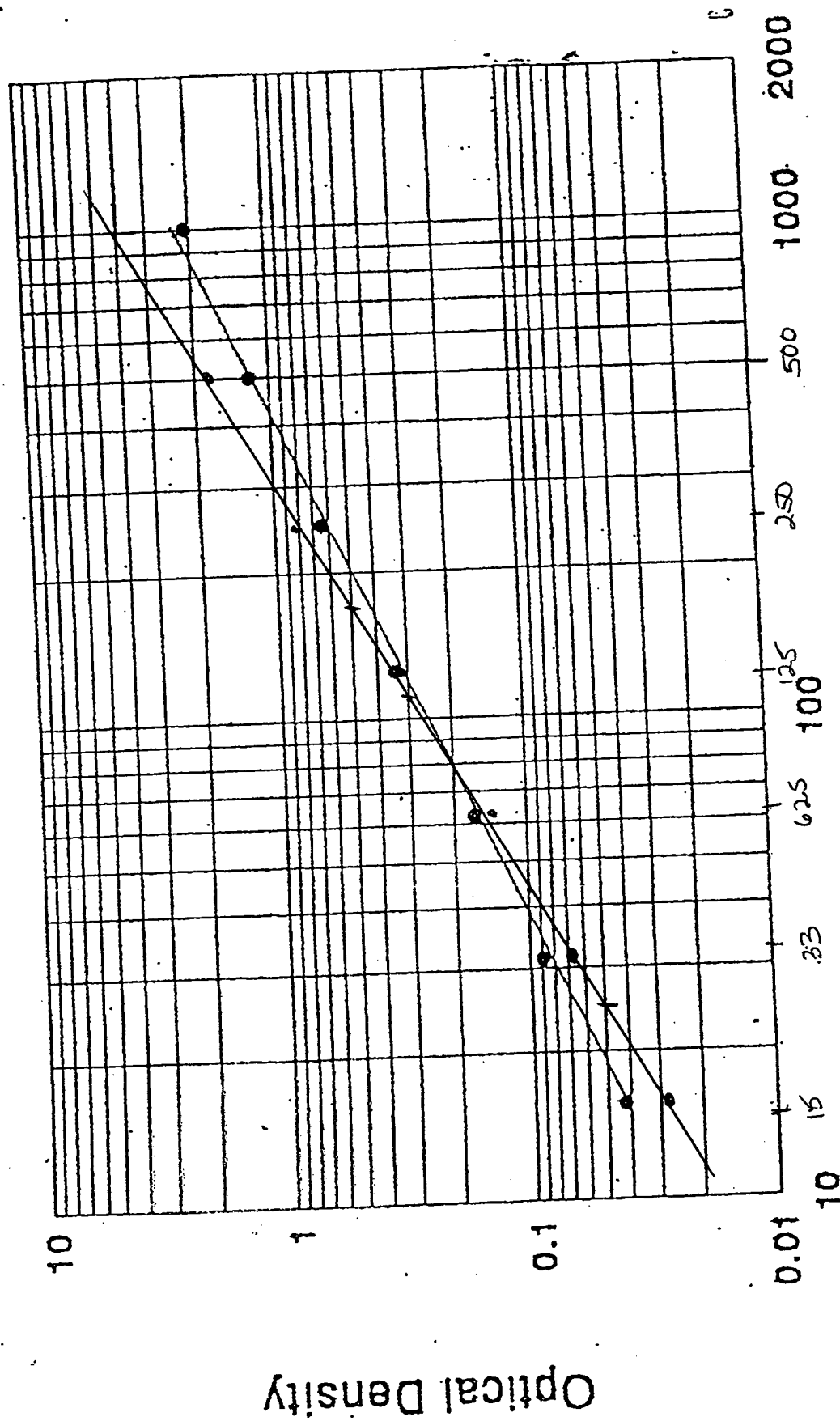
NOTES: _____ CAL: 0%

2616 499-6 DIVINS

OPTICAL DENSITY											
1	2	3	4	5	6	7	8	9	10	11	12
0.052	0.054	0.043	0.051	0.045	0.047	0.047	0.000	0.000	0.000	0.000	0.000
.028	.027	0.050									
0.040	0.078	0.045	0.053	0.045	0.048	0.048	0.000	0.000	0.000	0.000	0.000
.069	.141										
0.111	0.120	0.045	0.044	0.047	0.047	0.051	0.000	0.000	0.000	0.000	0.010
.161	.158										
0.213	0.237	0.044	0.051	0.047	0.072	0.039	0.000	0.000	0.000	0.000	0.000
.311	.326										
0.363	0.311	0.045	0.045	0.105	0.090	0.061	0.000	0.000	0.000	0.000	0.000
.878	.844										
0.927	1.895	0.045	0.047	0.228	0.101	0.047	0.000	0.000	0.000	0.000	0.000
1.777	1.851										
1.824	1.901	0.047	0.045	0.155	0.172	0.050	0.000	0.000	0.000	0.000	0.000
1.814											
		0.045	0.050	0.155	0.197	0.050	0.000	0.000	0.000	0.000	0.000

TNF- α Standard Curve

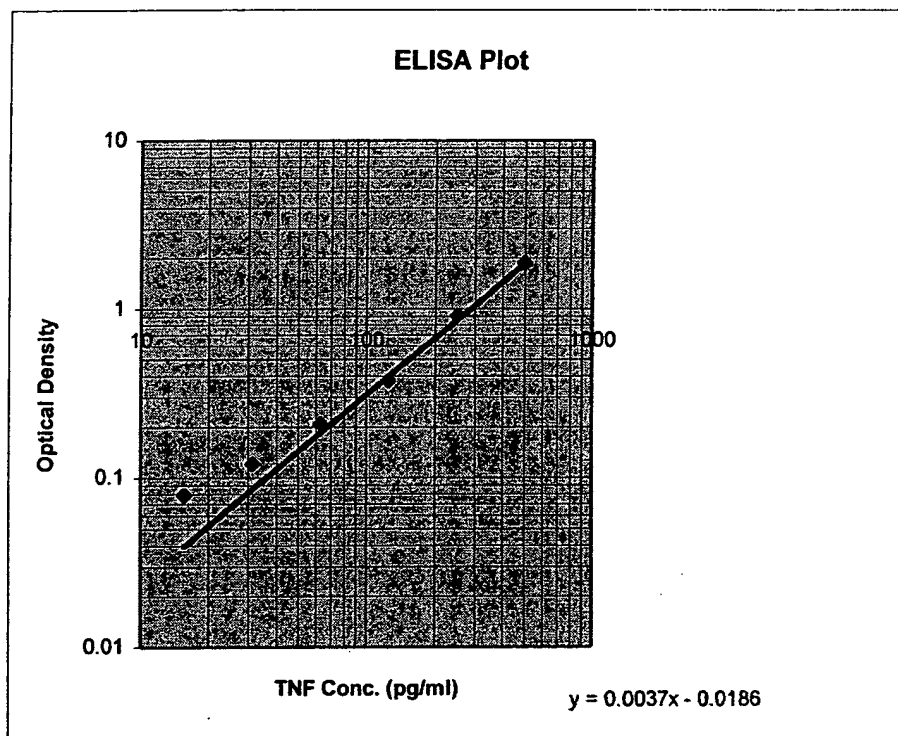
Diluent RD5



Concentration of TNF- α (pg/mL)

ELISA Plotting Worksheet

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
0.106	0.099	32.7	0.1025
0.101	0.101	32.3	0.101
0.172	0.172	51.5	0.172
0.185	0.197	56.6	0.191



T986: 899-6 ~~only~~ @ MOI 10^{-1}
RT 0, 2, 5, 20 [TNF]

STALEY

Purpose

- Quantity killing of T986 (glioblastoma cells) with R899-6 virus and 0, 2Gy, 5Gy & 20 Gy RT.
- Determine TNF production (? inducibility) with varying RT doses by ELISA.

Design

- Fixed Variables - Cell type: T986

~~RT~~

Virus: R899-6 @ MOI of 10^{-1}

Interval infection \rightarrow RT: 16 hrs

Interval RT \rightarrow subculture: 6 hrs

- Study variables -

RT dose: 0, 2Gy, 5Gy, 20Gy

Aliquot media for TNF: ^{prior to RT} 30 min, 1 hr, 2 hr, 4 hr

Virus: None, R899-6

$$4 \times 2 \times 2 (\text{dupl}) = 16 \text{ plates}$$

Methods - see

① Plated 5×10^5 T98G (Gibco) (Gibco)

② Plate count = 7.5×10^5 cells

③ Dilutions made

④ Change media to MEM + 2% + 1% NaPyruvate + 1% ess a.g. + 2% FCS

⑤ Add virus (3³⁰ pm)

for 10^{-1} 899-6 need

$$\frac{7.5 \times 10^5 \text{ cells PFU}}{6 \times 10^3 \text{ PFU/ml}} = 12.5 \text{ ul}$$

⑥ Wait 2 hrs, add 2 more ml of 2% media 5³⁰ pm

⑦ Irradiate 6⁴⁵ - 7¹⁵

⑧ Aliquot 0.5 ml media @ 30 mins = 7³⁰

@ 1 hr = 8¹⁵

@ 2 hr = 9¹⁵

@ 4 hr = 11¹⁵

⑨ Subculture @ 6 hr = 1¹⁵ (Don't subculture 20Gy plates)

counts: 899-6

0Gy =

2Gy =

5Gy =

20Gy =

Drains

0Gy =

2Gy =

5Gy =

20Gy =

(Save 1 ml of supernatant & pellet for TNF)

⑩ Plate @ 10^3 , 5×10^3 , 10^4

	1	2	3	4	5	6	7	8	9
1	TNF ELISA RESULTS								
2	(Note: error in standards, old std curve used)								
3									
4	Cell type: T986 (Gibco/Corning)								
5	Virus: R899-6 @ 10^6 cfu/ml								
6									
7	RT	Aliquot Time	Virus	[TNF]	Controls (No Virus)				
8	0 Gy	30'	899-6	N/A	0 Gy } 2 Gy } No TNF 5 Gy } 20 Gy }				
9		1°		N/A					
10		2°		49					
11		4°		64					
12		6°		94					
13		Pellet (10^5 /ml)		23 = $230 @ 10^6$ /ml					
14	2 Gy	30'	899-6	59					
15		1°		67					
16		2°		(115)					
17		4°		78					
18		6°		73					
19		Pellet (10^5 /ml)		25 = $250 @ 10^6$ /ml					
20	5 Gy	30'	899-6	46					
21		1°		50					
22		2°		54					
23		4°		70					
24				101					
25		Pellet (10^5 /ml)		24 = $240 @ 10^6$ /ml					
26	20 Gy	30'	899-6	56					
27		1°		83					
28		2°		66					
29		4°		82					
30		6°		127					
31		Pellet (10^5 /ml)		27 = $270 @ 10^6$ /ml					

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
1° 0.181	0.181	53.9	0.181
0.143	0.143	43.7	0.143
2° 0.226	0.24	68.0	0.233
0.2	0.201	59.2	0.2005
4° 0.291	0.293	83.9	0.292
0.369	0.362	103.8	0.3655
6° 0.066	0.067	23.0	0.0665
0.069	0.063	22.9	0.066
0.243	0.253	72.1 30'	0.248
0.152	0.143	44.9	0.1475
0.157	0.167	48.8 1°	0.162
0.303	0.287	84.8	0.295
0.532	0.517	146.8 2	0.5245
0.175	0.173	52.1 6	0.174
0.314	0.336	92.9 6	0.325
0.203	0.195	58.8 4	0.199
0.345	0.342	97.9 4	0.3435
0.293	0.286	83.3 2	0.2895
0.062	0.067	22.5	0.0645
0.076	0.086	26.9	0.081

0.157
0.142
0.148
0.191
0.183
0.177
0.234
0.231
0.346
0.371
0.063
0.064

0.182
0.17
0.208
0.348
0.246
0.205
0.261
0.301
0.457
0.456
0.083
0.085

0.158
0.146
0.151
0.179
0.192
0.17
0.251
0.238
0.337
0.36
0.075
0.073

0.203
0.193
0.247
0.358
0.248
0.209
0.273
0.306
0.448
0.437
0.079
0.079

47.6
43.9
45.4
55.0
55.7
51.9
70.6
68.4
97.3
103.8
23.7
23.5

57.1
54.1
66.5
100.4
71.8
61.0
77.2
87.1
127.3
125.7
26.9
27.2

0.1575
0.144
0.1495
0.185
0.1875
0.1735
0.2425
0.2345
0.3415
0.3655
0.069
0.0685

0.1925
0.1815
0.2275
0.353
0.247
0.207
0.267
0.3035
0.4525
0.4465
0.081
0.082

[illegible]

MOLECULAR DEVICES

Umax KINETICS MICROPLATE READER

PLATE #1

ASSAY : _____

DATE: _____

OPERATOR: _____ O.D. LIMIT: 3.000

TIME: _____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

AUTO MIX: OFF

NOTES: _____ CAL: 0

OPTICAL DENSITY

	13	14	15	16	5	6	7	8	9	10	11	12
-2°												
A	0.246	0.246	0.053	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.205	0.209	0.053	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
-4°												
C	0.261	0.273	0.059	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.301	0.306	0.058	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
-6°												
E	0.457	0.448	0.056	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.458	0.427	0.055	0.056	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
-P												
G	0.082	0.079	0.056	0.055	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000
H	0.025	0.075	0.059	0.057	0.001	0.001	0.000	0.000	0.000	0.001	0.000	0.000

MOLECULAR DEVICES

Umax KINETICS MICROPLATE READER

PLATE #1

ASSAY : _____

DATE: _____

OPERATOR: _____ O.D. LIMIT: 3.000

TIME: _____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

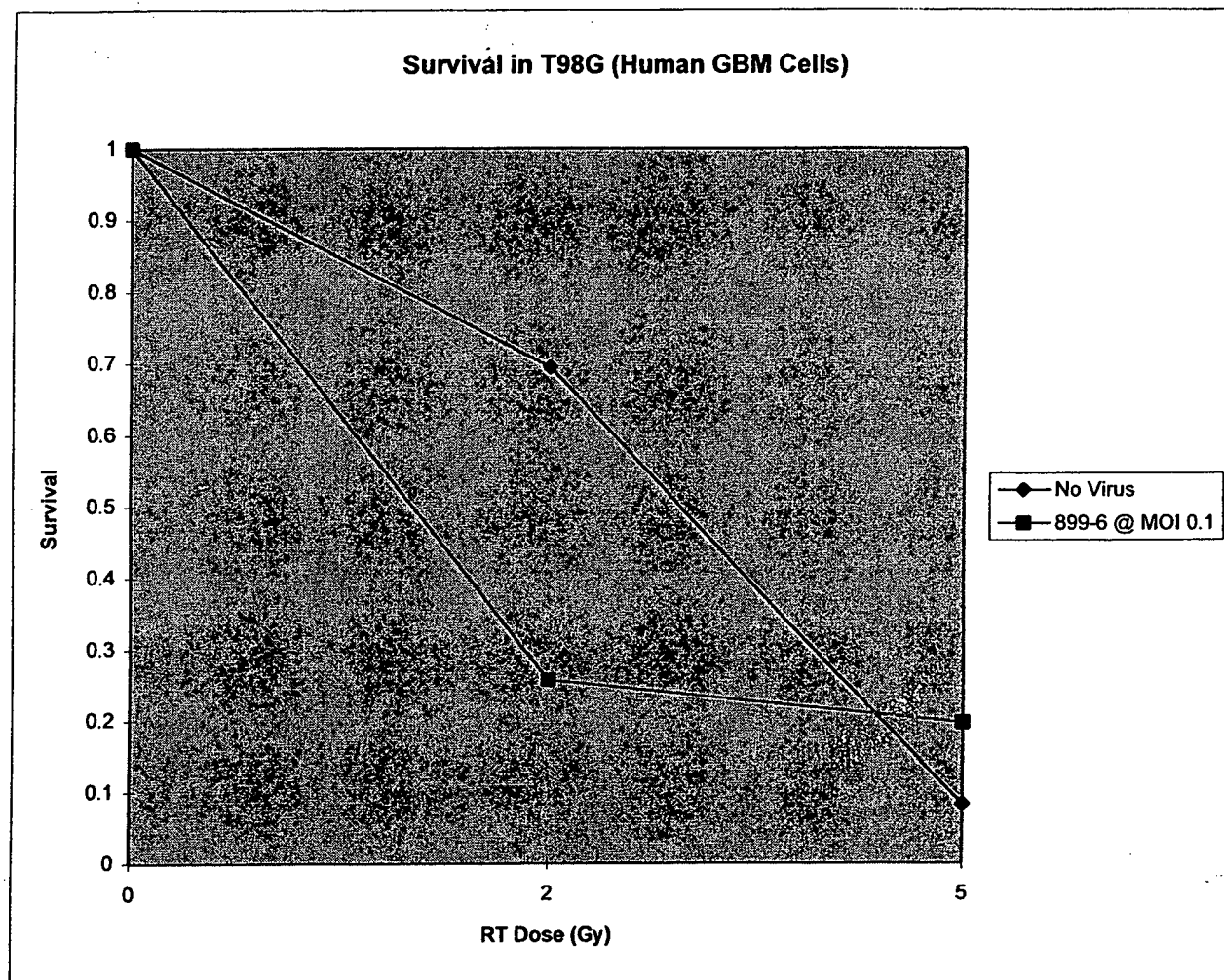
AUTO MIX: OFF

NOTES: _____ CAL: 0

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
			30'		6°				30'	56°	6°	
1	0.002	0.000	0.000	0.003	0.221	0.292	0.522	0.527	0.207	0.135	0.345	0.217
2	0.008	0.006	0.007	0.008	0.382	0.362	0.770	0.473	0.142	0.140	0.371	0.380
3	0.004	0.001	10'	0.007	0.004	0.056	0.057	0.214	0.230	0.108	0.151	0.082
4	0.000	0.007	0.003	0.007	0.059	0.052	0.203	0.192	0.191	0.175	0.066	0.072
5	0.002	0.005	0.051	0.181	0.243	0.253	0.345	0.342	0.183	0.192	0.188	0.202
6	0.001	0.004	0.065	0.143	0.152	0.143	0.293	0.165	0.177	0.170	0.170	0.153

h/ho



Results- Count Plates T98G Cells

RT	Virus	# Plated	# Counted	#Counted	Average	P.E.	Survival	
0	0	1000	154	104	0.129	0.129	1	
		5000	tm	tm				
		10000	tm	tm				
2	0	1000	75	104	0.0895		0.693798	
		5000	tm	tm				
		10000	tm	tm				
5	0	1000	18	13	0.0155		0.120155	0.081783
		5000	20	44	0.0064		0.049612	
		10000	71	124	0.00975		0.075581	
0	899-6	1000	12	7	0.0095	0.015267	1	
	(MOI=0.1)	5000	68	139	0.0207			
		10000	156	156	0.0156			
2	899-6	1000	3	6	0.0045		0.294118	0.255991
		5000	13	17	0.003		0.196078	
		10000	25	60	0.00425		0.277778	
5	899-6	1000	4	2	0.003		0.196078	0.196078
		5000	18	11	0.0029		0.189542	
		10000	38	24	0.0031		0.202614	
RT Dose	No Virus	899-6 @ MOI 0.1						
0	1	1						
2	0.694	0.256						
5	0.082	0.196						

1	2	3	4	5	6	7	8	9	
RESULTS - Count plates									
RT	Virus	# plated	# counted 1	# counted 2	Counted ave.	P.E.	SURVIVAL	Ave SURVIVAL	
Ø	Ø	10^3	154	104	129	.13	100	100	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
2 Gy	Ø	10^3	75	104	90	"	69	69	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
5 Gy	Ø	10^3	18	13	16	"	12	8.1	
		5×10^3	20	44	32	"	4.8		
		10^4	71	124	98	"	7.5		
					1.7	.017			
Ø	899-6	10^3	12	7	9.5	"	7.3	11.7	
		5×10^3	68	139	103	"	15.9		
		10^4	Tm	156	156	"	12		
2 Gy	899-6	10^3	3	6	4.5		3.5	4.3	.17
		5×10^3	13	17	15	"	2.3		
		10^4	25	(160)	25 (92.5)	"	1.9 (7.1)		
5 Gy	899-6	10^3	2	4	3	"	2.3	2.3	
		5×10^3	18	11	14.5	"	2.2		
		10^4	38	24	31	"	2.4		

FINDINGS -

- ① R899-6 virus is highly cytotoxic at an MOI of 10^{-1} to T98G cells with a plating efficiency of only 1.5%.
- ② Higher levels of TNF are produced in T98G cells than in SQ20B cells = 5 fold more at an MOI of 10^{-1} (250 vs 50).
- ③ No radiation induction of TNF, i.e. it is produced constitutively.
- ④ There is no clear synergistic effect between 5 Gy & 899-6 @ 10^{-1} , although there is possible synergy at 2 Gy.
- ⑤ T98G cells are more radiosensitive than SQ20B cells.

SQ 20B

Purpose-

- Quantify cell killing of R3616 at MOI of 10^{-2} , 10^{-1} , 5.
- Is killing additive or synergistic \bar{c} 5 Gy?
- Quantify the effect of IgG (human) in the incubation media
- Does the IgG Is there a cell density dependent killing, and does IgG eliminate this phenomenon

Design

- Fixed Variables - Cell type: SQ 20B
Virus: R3616
Interval from infection: 2° then
Interval from infection to RT: 16°
Interval from RT to subcult: 4-6°
- Study Variables - Virus: None, R3616 @ MOI 10^{-2} , 10^{-1} , 5
Human IgG: +, -
RT: 0 Gy, 5 Gy

5Q-20B

EFFICIENCY LINE® 22-206

	1	2	3	4	5	6	7	8	9
1	Virus	mol	IgG	RT					
2	Ø	—	0	0	5.6				
3	"	"	"	"	4.8				
4	Ø	—	+	0	6				
5	Ø	—	0	5	3.4				
6	"	"	"	"	3.7				
7	Ø	—	+	5	4.2				
8	3616	10^{-2}	0	0	4.2				
9	"	"	"	"	inf.				
10	3616	10^{-2}	+	0	5.9				
11	"	"	"	"	4.2				
12	3616	10^{-2}	0	5	2.7				
13	"	"	"	"	2.4				
14	3616	10^{-2}	+	5	2.8				
15	"	"	"	"	4.2				
16	3616	10^{-1}	0	0	5.9				
17	"	"	"	"	5.8				
18	3616	10^{-1}	+	0	3.3				
19	"	"	"	"	3.35				
20	3616	10^{-1}	0	5	2.2				
21	"	"	"	"	1.9				
22	3616	10^{-1}	+	5	1.8				
23	"	"	"	"	1.8				
24	3616	5	0	0	1.8				
25	"	"	"	"	2.5				
26	3616	5	+	0					
27	"	"	"	"					
28	3616	5	0	5					
29	"	"	+	"					
30	3616	5	+	5					
31	"	"	"	"					

switch

need 31 plates \times 5 ml = 155 ml
 \therefore resuspend in 165 ml
 10^7 cells in 165 ml = 3×10^5 / 5 ml

	1	2	3	4	5	6	7	8	9	
1										
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5										
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31										

Methods -

① Plate 5×10^5 cells per 60 mm dish

② Infect \bar{c} virus in 2 ml 2% FCS

Dilutions: Stock 10^6 10^5 10^4 10^3

5×10^6 PFU/ml 6×10^5 6×10^4 6×10^3

Count dish = 2×10^6 cells

- MOI 10^{-2} = 2×10^4 PFU/ml = 3.3 ul of 6×10^3 dilution

- MOI 10^{-1} = 2×10^5 cells 6×10^4 PFU/ml = 3.3 ul of 6×10^4 dilution

- MOI 5 = 1×10^7 PFU/ml = 16.7 ul of 6×10^5 dilution

③ Incubate 2° then ~~add~~ aspirate media &

add: 4 ml of 2% FCS (\ominus IgG) or

4 ml of 2% FCS + human γ glob (1 ul/ml) (stock = $165 \text{ mg}/10 \text{ ml}$)

④ Irradiate \bar{c} 0 or 5 Gy

⑤ Subculture & count each dish \bar{c} hemacytometer

⑥ Make dilutions 10^6 , 10^5 , 10^4 , 10^3 for each dish

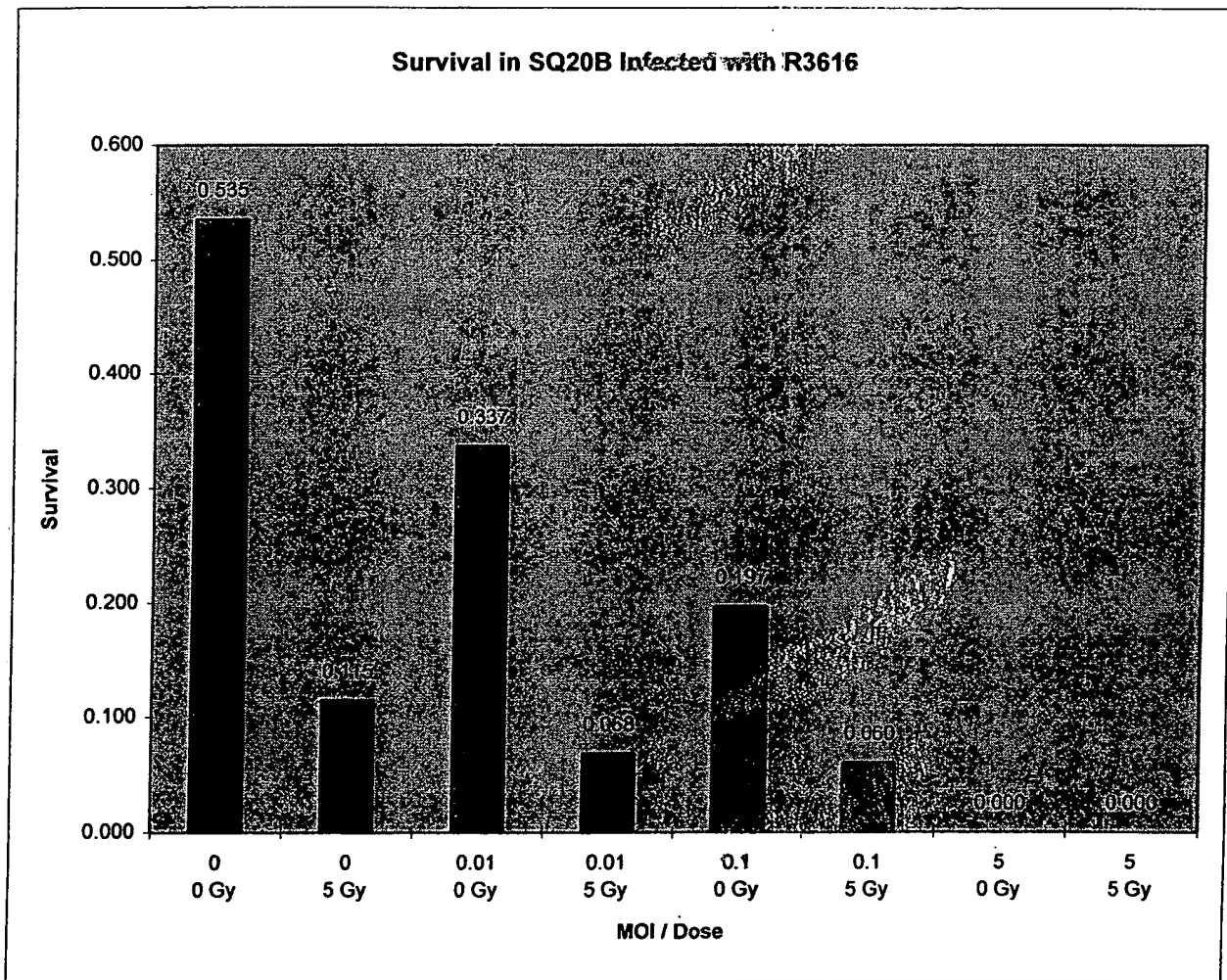
⑦ Plate 3 dilutions for each original dish = 5×10^2 , 10^3 , 5×10^3

⑧ Incubate for 10 d - 14 d

SQ 20B: R3616 BY MOI AND +/- IgG								
Virus	MOI	IgG	RT	# plated	# counte	Crude SV	PE	Rel. SV
0	-	-	0	500	323	0.646	0.535333	100
0	-	-	0	500	203	0.406		
0	-	+	0	500	277	0.554		
0	-	-	5	500	36	0.072	0.533	0.214822
0	-	-	5	500	72	0.144		
0	-	+	5	500	56	0.112		
0	-	+	5	1000	140	0.14		
0	-	-	5	1000	89	0.089		
0	-	-	5	1000	130	0.13		
R3616	0.01	-	0	500	214	0.428	0.3366	100
R3616	0.01	-	0	500	130	0.26		
R3616	0.01	+	0	500	177	0.354		
R3616	0.01	+	0	500	205	0.41		
R3616	0.01	-	0	1000	231	0.231		
R3616	0.01	-	5	500	49	0.098	0.337	0.202733
R3616	0.01	-	5	500	43	0.086		
R3616	0.01	+	5	500	25	0.05		
R3616	0.01	+	5	500	48	0.096		
R3616	0.01	-	5	1000	90	0.09		
R3616	0.01	-	5	1000	68	0.068		
R3616	0.01	+	5	1000	47	0.047		
R3616	0.01	+	5	1000	49	0.049		
R3616	0.01	+	5	5000	205	0.041		
R3616	0.01	+	5	5000	287	0.0574		
R3616	0.1	-	0	500	96	0.192	0.197	
R3616	0.1	+	0	500	101	0.202		
R3616	0.1	+	0	500	[251]	-		
R3616	0.1	-	0	1000	TM	-		
R3616	0.1	+	0	1000	197	0.197		
R3616	0.1	+	0	1000	TM	-		
R3616	0.1	-	5	500	51	0.102	0.197	0.305922
R3616	0.1	-	5	500	25	0.05		
R3616	0.1	+	5	500	31	0.062		
R3616	0.1	+	5	500	38	0.076		
R3616	0.1	-	5	1000	68	0.068		
R3616	0.1	-	5	1000	55	0.055		
R3616	0.1	+	5	1000	59	0.059		
R3616	0.1	+	5	1000	77	0.077		
R3616	0.1	-	5	5000	215	0.043		
R3616	0.1	-	5	5000	185	0.037		
R3616	0.1	+	5	5000	246	0.0492		
R3616	0.1	+	5	5000	225	0.045		
R3616	5	+	0	10000	0	0	0	0
R3616	5	+	5	10000	0	0		

SQ 20B: R3616 BY MOI AND +/- IgG

Dose	MOI	Survival
0 Gy	0	0.535
5 Gy	0	0.115
0 Gy	0.01	0.337
5 Gy	0.01	0.068
0 Gy	0.1	0.197
5 Gy	0.1	0.060
0 Gy	5	0.000
5 Gy	5	0.000





	1	2	3	4	5	6	7	8	9	
1										
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FINDINGS

In SQ-208:

- There is 63% & 37% survival @ MOI of 10^{-2} & 10^{-1} (corrected for plating efficiency)
- 5 Gy radiation results in 21% survival 5 virus (corrected for plating efficiency)
- Cell killing is additive between virus and radiation at the MOI & dose studied.
- An MOI of 5 results in complete cell killing
- Human γ -globulin in the ^{medium} serum did not have an effect on survival
- No cell density dependence was observed

V 87 cells

	1	2	3	4	5	6	7	8	9
1									
2		<u>Purpose -</u>							
3		- Compare cytotoxicity of R3616 & R899-6 in							
4		U-87 cells (human glioblastoma).							
5		- Determine radiosensitivity of U-87 cells to 5 Gy							
6		- Are Is viral cytotoxicity additive or synergistic							
7		with radiation toxicity?							
8		- Quantitate TNF production in R899-6 cells.							
9		Does RT (5 Gy) increase TNF production?							
10									
11									
12									
13									
14									
15									
16									
17		<u>Design -</u>							
18		- Fixed variables - cell type: U-87 (human glioblastoma)							
19									
20									
21		Study variables -							
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

V:vs: None, R3616, R899-6
 MOI: 10^{-3} , 10^{-1}
 RT: 0, 5 Gy

U-87 cells

EFFICIENCY LINE 22-206



	1	2	3	4	5	6	7	8	9
1	Virus	MOI	RT	INF assay					
2	Ø	—	0	P only					
3	"	—	0	no					
4	"	—	5	P only					
5	"	—	5	no					
6	3616	10^{-1}	0	no					
7	"	"	"	no					
8	3616	10^{-1}	5	no					
9	"	"	"	no					
10	3616	10^3	0	P only					
11	"	"	"	no					
12	3616	10^3	5	P only					
13	"	"	"	no					
14	899-6	10^{-1}	0	Y					
15	"	"	"	Y					
16	899-6	10^{-1}	5	Y					
17	"	"	"	Y					
18	899-6	10^{-3}	0	Y					
19	"	"	"	Y					
20	899-6	10^{-3}	5	Y					
21	"	"	"	Y					
22	Extra plate to count								
23									
24	∴ need 21 plates x 5 ml = 105 ml								
25	resuspend cells in 110 ml								
26									
27	Plate 10^5 , i.e. 2.2×10^6 cells in 110 ml								
28									
29									
30									
31									

Need 6 plates = Ø virus

3616 10^{-1}

3616 10^{-3}

899-6 10^{-1}

899-6 10^{-1}

count

I didn't do this.
No INF assays done

V87 cells

Methods

- ① Plate 10^5 cells per 60 mm plate. Need 6 plates
- ② When >90% confluent, infect w/ virus. Count cells in extra plate to determine PFU's.
Make dilutions of virus = 6×10^6 PFU/ml (stock) $\rightarrow 6 \times 10^5 \rightarrow 6 \times 10^4 \rightarrow 6 \times 10^3$
Count dish = 2.65×10^6
- mol $10^{-1} = \frac{2.65 \times 10^6 \text{ PFU}}{2.65 \times 10^3 \text{ PFU/ml}} = 4.4 \text{ ml}$
- mol $10^{-3} = \frac{2.65 \times 10^6 \text{ PFU}}{6 \times 10^2 \text{ PFU/ml}} = 4.4 \text{ ml}$
- ③ Incubate 2° in 20% FCS
dilutions = $10^6, 10^5, 10^4, 10^3$ cells/ml, 10^2
- ④ Plate dilutions:
Ø virus, Ø RT = 200, 500, 1000
Ø virus, 5% Gy = $5 \times 10^3, 10^4, 2 \times 10^4$
virus, Ø RT = $5 \times 10^2, 10^3, 5 \times 10^3, 10^4$
virus, 5% Gy = $5 \times 10^3, 10^4, 5 \times 10^4, 10^5$
Media = 10% FCS + 1% NaPyr + 1% Ess a.a. in MEM $\pm 8.966 \text{ } \mu\text{g/ml}$
- ⑤ Wait 4° , then irradiate
- ⑥ Incubate
- ⑦ Stain & count

U-87

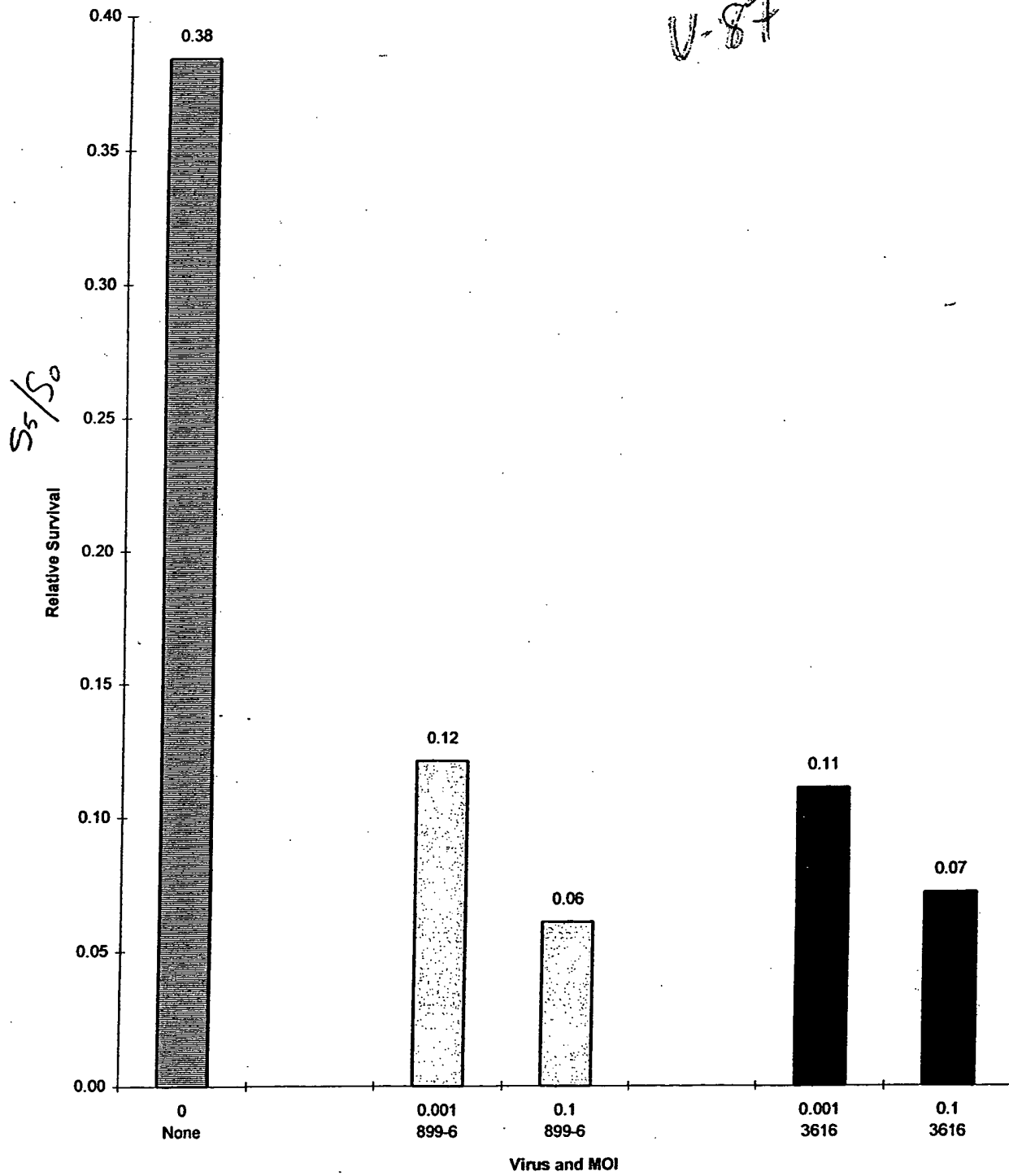
Note: poorly defined plaques, slow growing

EFFICIENCY LINE # 22-206

	1	2	3	4	5	6	7	8	9
1	<u>RESULTS -</u>								
2	VINS	MOI	RT	# plated	# tanted	Crude SV	PE	Re/SV	
3	0	-	0	200	0/0				
4				500	0/1				
5				1000	6				
6	0	-	5	5000	1/12				
7				10 ⁴	0/12				
8				2x10 ⁴	42/45				
9	899-6	10 ⁻³	0	1000	21/6				
10				5000	61				
11				10 ⁴	?/1?				
12	899-6	10 ⁻³	5	5000	1/1				
13				10 ⁴	0/7				
14				5x10 ⁴	?/106				
15	899-6	10 ⁻¹	0	1000	35/6				
16				5000	125/69				
17				10 ⁴	?/1?				
18	899-6	10 ⁻¹	5	5000	1/0				
19				10 ⁴	6/8				
20				5x10 ⁴	138/148				
21	3616	10 ⁻³	0	1000	3/0				
22				5000	68/43				
23				10 ⁴	?/?				
24	3616	10 ⁻³	5	5000	0/1				
25				10 ⁴	12/14				
26				5x10 ⁴	?/?				
27	3616	10 ⁻¹	0	1000	14/8				
28				5000	106/?				
29				10 ⁴	?/?				
30	3616	10 ⁻¹	5	5000	1/0				
31				10 ⁴	4/22				
				5x10 ⁴	?/104(?)				

Survival at 5 Gy Relative to Survival at 0 Gy

V-87

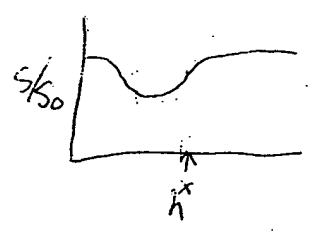
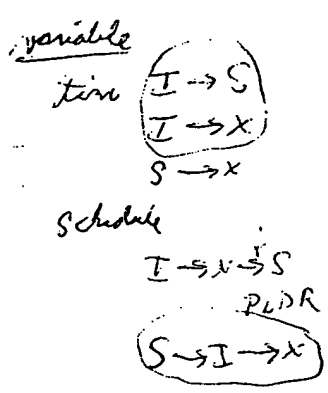
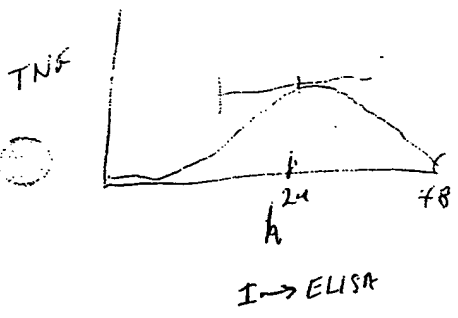


U-87									
RESULTS:									
Virus	MOI	RT	# plated	# counted		Crude SV	Ave SV	PE	Rel SV
None	-	0	200	0	0	0	0.002667	0.002667	1
None	-	0	500	1		0.002			
None	-	0	1000	6		0.006			
None	-	5	5000	1	2	0.0003	0.001025	0.002667	0.384375
None	-	5	10,000	0	12	0.0006			
None	-	5	20,000	42	45	0.002175			
899-6	0.001	0	1000	21	6	0.0135	0.00735	0.00735	1
899-6	0.001	0	5000	6		0.0012			
899-6	0.001	0	10,000	tm	tm				
899-6	0.001	5	5000	1	1	0.0002	0.00089	0.00735	0.121088
899-6	0.001	5	10,000	0	7	0.00035			
899-6	0.001	5	50,000	106	tm	0.00212			
899-6	0.1	0	1000	35	6	0.0205	0.01995	0.01995	1
899-6	0.1	0	5000	125	69	0.0194			
899-6	0.1	0	10,000	tm	tm				
899-6	0.1	5	5000	0	1	0.0001	0.00122	0.01995	0.061153
899-6	0.1	5	10,000	6	8	0.0007			
899-6	0.1	5	50,000	138	148	0.00286			
3616	0.001	0	1000	3	0	0.0015	0.0063	0.0063	1
3616	0.001	0	5000	68	43	0.0111			
3616	0.001	0	10,000	tm	tm				
3616	0.001	5	5000	0	1	0.0001	0.0007	0.0063	0.111111
3616	0.001	5	10,000	12	14	0.0013			
3616	0.001	5	50,000	tm	tm				
3616	0.1	0	1000	14	8	0.011	0.0161	0.0161	1
3616	0.1	0	5000	106	tm	0.0212			
3616	0.1	0	10,000	tm	tm				
3616	0.1	5	5000	1	0	0.0001	0.00116	0.0161	0.07205
3616	0.1	5	10,000	4	22	0.0013			
3616	0.1	5	50,000	104	tm	0.00208			

1/2

?

?



SQ20B

Purpose

- Determine viral cytotoxicity of R3616 @ MOI of 10^{-1} & 1 in SQ20B cells
- Is viral cell killing additive or synergistic with 9 Gy RT?

Design

- Fixed variables - Cell Type: SQ20B

~~Virus~~
Interval infection \rightarrow subculture: 2 hours
Interval subculture \rightarrow RT: 4 hours
Human γ globulin added to plates
P subculture

- Study variables - Virus: R3616 @ MOI 0, 10^{-1} , 1
RT: 0, 9 Gy

	1	2	3	4	5	6	7	8	9
1	TITERS								
2									
3	Cell	Virus	Dilution	Plaque #	Titer				
4	SQ 20B	3616	10^7	0	?				
5			"	0					
6			10^8	0					
7			"	0					
8	SQ 20B	899-6	10^7	0	?				
9			"	0					
10			10^8	0					
11			"	0					
12	V-87	3616	10^7	32 13	6×10^7				
13			"	42 8					
14			10^8	8 0					
15			"	7 0					
16	V-87	899-6	10^7	33 13	1.1×10^8				
17			"	42 8					
18			10^8	8 0					
19			"	7 0					
20	T-986	3616	10^7	14	1.2×10^8				
21			"	9					
22			10^8	0					
23			"	0					
24	T-986	899-6	10^7	12	4.1×10^8				
25			"	39					
26			10^8	3	8×10^8				
27			"	7					
28									
29									
30									
31									

none seen, but cells overgrown.
 by filtering @ 80%
 confluence

small
 plaques

nice
 plaques

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 18773

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

☐ STANDING ORDER:

SHIPMENTS ON A _____ BASIS

DATE: _____

REQUEST BY: Craig Sibley, MDREQUESTORS PHONE NUMBER: 312-702-0284AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 6-95150-5100VENDOR: Jackson LabsREQUESTED DELIVERY DATE: ASAPPI: HallahanPROTOCOL: 58671PHONE: 2-689SPECIES: mouseQUANTITY: 24SEX: M F EITHERWEIGHT/AGE: 5-7 wks

ALTERNATE WEIGHT/AGE: _____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

SPECIAL REQUIREMENTS: _____

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

HOUSE AT: _____ CARLSON _____ WYLER _____ CLSC _____ FMI _____ OTHER _____

PROCUREMENT DESK: 2-8364

T986

Purpose -

- Establish dose response survival curves with T986 cell line. ~~Dose~~
- ~~Establish dose response survival curve with T986 cell line + ~~EA~~ R3616 @ MOI = 10^{-1} .~~

Design -

- Fixed variables - cell type T986 (human glioblastoma)
 - ~~intervals - infect x 2° → subculture → wait 4° → irradiate~~
- Study variables - v:ns: None, ~~R3616 @ MOI = 10^{-1}~~
 Rt dose: 0, 2, 3, 5, 7, 9, 11 Gy

METHODS

① Plate 7986 cells on 60 mm dish & grow to ~90% confluent
Need 5 plates (Plate 10^5 /dish & 5×10^4 /dish = 10 dishes
& choose best set) 2 plates = 13616

② Count 1 plate & infect ~~plates~~ @ $MOI = 10^{-1} = 199V \times 2^0$
Count = 1.6×10^6 cells, $MOI 10^{-1} = \frac{1.6 \times 10^6}{6 \times 10^4 \text{ PFU/ml virus dilution}} = 2.7 \text{ AL}$
* I infected @ $MOI = 3.75$ by mistake

③ After 2^o infection subculture to 100 mm plates (in duplicate)

RT	Virus	# plated
0	-	200 500 1000
2	-	200 500 1000
3	-	500 1000 2000
5	-	2000 5000 10^4
7	-	10^4 5000 10^4
9	-	10^4 10^5

1	+	2000	5000
2	+	5000	10^4
3	+	5000	2×10^4
5	+	10^4	5×10^4
7	+	5×10^4	10^5
9	+	10^5	270

∴ can't use

EFFICIENCY LINE# 22-206

Conclusions

① Poor plotting efficiency. Can't draw any conclusions. Repeat.

TITER ~~CELLS~~ VIRUSES IN 4 CELL LINES

[illegible]

[illegible]

	1	2	3	4	5	6	7	8	9
1	<u>In-vitro - SQ-20B</u>								
2	1.) Titer virus in SQ-20B								
3	2.) Generate Survival curve for R3616 @ 0, 3, 5, 7, 9, 11 Gy - MOI=0								
4									
5	<u>- U87</u>								
6	1.) Titer virus in U-87								
7	2.) Generate Survival curve for R3616 (MOI=10 ⁻³) @ 0, 2, 3, 5, 7 Gy								
8	R3616 (MOI=10 ⁻¹)								
9	R899-6 (MOI=10 ⁻³)								
10	R899-6 (MOI=10 ⁻¹)								
11									
12	<u>- T98G</u>								
13	1.) Titer virus in U-87								
14	2.) Generate survival curves as above for U87								
15									
16									
17									
18									
19									
20	<u>TNF</u>								
21	1.) Measure in U-87 & T98G infected w/ R3616 to								
22	R/O cellular TNF production in response to virus								
23	2.) Quantify								
24									
25									
26									
27									
28									
29									
30									
31									



	1	2	3	4	5	6	7	8	9
1	<u>TITERS</u>								
2	Cell	Virus	Dilution	Plaque	ct	Titer			
3	Vero	3616	10 ⁶	Tm	Tm				
4			10 ⁷	230	bad flask				
5			10 ⁸	23	24	2.3 x 10 ⁹			
6	Vero	899-6	10 ⁶						
7			10 ⁷	104	117				
8			10 ⁸	15	12	1.2 x 10 ⁹			
9	T986	3616	10 ⁵						
10			10 ⁶	35x					
11			10 ⁷	12					
12	T986	899-6	10 ⁵						
13			10 ⁶	171					
14			10 ⁷	19					
15	V87	3616	10 ⁵						
16			10 ⁶						
17			10 ⁷						
18	V87	899-6	10 ⁵						
19			10 ⁶						
20			10 ⁷						
21	SQ203	3616	10 ⁴						
22			10 ⁵						
23			10 ⁶						
24	SQ203	899-6	10 ⁴						
25			10 ⁵						
26			10 ⁶						
27									
28									
29									
30									
31									

10²
10³
10⁴
10⁵
10⁵
10⁶

U-87

PURPOSE -

- Establish dose response survival curve with U87 cell line
- Establish dose response survival curve with U87 cell line + R366 @ $MOI = 10^{-1}$

DESIGN -

- Fixed variables - cell type U87 (human glioma)
- intervals = infect $\times 2^{\circ} \rightarrow$ subcult $\rightarrow 4^{\circ} \rightarrow$ XRT
- Study variables - virus: None, R366 @ $MOI = 10^{-1}$
- RT dose: 0, 2, 3, 5, 7, 9 Gy

U-87

METHODS

- ① Plate U87 cells on 60 mm dish & grow to ~80% confluence. Need 5 plates. (plate 5×10^5 /plate)
- ② Count 1 plate & infect 2 plates @ 10^{-1} (R3616) for 2°
 $1.2 \times 10^6 \text{ cells} \times 10^{-1} (\text{moi}) = 1.2 \times 10^5 \text{ PFU}$
 $\frac{1.2 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/ml dil.}} = 2 \text{ ml} \times 8 \text{ ml}$

- ③ After 2° infection, subculture to 100mm plates (in duplicate)

RT	Virus	# plated		
0	-	500	1000	5000
2	-	1000	5000	10^4
3	-	5000 10^4	10^4	2×10^4
5	-	10^4	2×10^4	
7	-	10^4	5×10^4	10^5
9	-	5×10^4	10^5	
0	+	1000	2000	5000
2	+	5000	10^4	
3	+	10^4	2×10^4	
5	+	10^4	2×10^4	5×10^4
7	+	5×10^4	10^5	
9	+	5×10^4	10^5	

- ④ Stain & count

EFFICIENCY LINE# 22-206

[illegible]

EFFICIENCY LINE 22-206

[illegible]

V-87

EFFICIENCY LINE # 22-206

CONCLUSIONS

- ① V87 is a fairly radioresistant cell line
(surviving cells @ 9 Gy)
- ② Does not appear to be a major impact
of R3616 on dose response curve.
- ③ Plating efficiency extremely low ($= 5 \times 10^{-4}$)
Cannot draw any quantitative conclusions
- ④ Sit estimates SF₂ (in vitro) at 0.5.
- ⑤ Repeat using conditioned media

[illegible]

METHODS -

- ① Plate V87 cells on 3 60mm dishes = 5×10^5
- ② ~~Count~~ Grow to subconfluence, Count 1 dish to calculate MOI, Count = 3×10^6 cells

New 888-6 liter = 1.2×10^9 PFU/ml

For MOI 10^{-1} : 3×10^5 PFU / 1.2×10^9 PFU/ml d.i. = ~~25000~~ 25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

For MOI 10^{-3} : 3×10^3 PFU / 1.2×10^9 PFU/ml d.i. = ~~2500~~ 25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

- ③ Infect x 2° then subcult to 100mm plates (in dupl,

RT	Virus	# plates		
0	10^{-1}	1000	2000	5000
2	10^{-1}	5000	10^4	
3	10^{-1}	10^4	2×10^4	
5	10^{-1}	10^4	2×10^4	
7	10^{-1}	5×10^4	10^5	
9	10^{-1}	5×10^4	10^5	
0	10^{-3}	1000	2000	5000
2	10^{-3}	5000	10^4	
3	10^{-3}	10^4	2×10^4	
5	10^{-3}	10^4	2×10^4	
7	10^{-3}	5×10^4	10^5	
9	10^{-3}	5×10^4	10^5	

0	Ø	500	1000	5000
---	---	-----	------	------

- ④ Stain & count (perfect size to count)

V-87

RESULTS -

1	2	3	4	5	6	7	8	9
899-6 LANDS	RT	# P/ctd	# Canted		Ave Surv.	P.E.	rel Surv.	
10 ⁻¹	0	1000	0	—	—	.008	1	
		2000	1	1	—			
		5000	23	57	.008			
	2	5000	11	3	.0045		.56	
		10 ⁴	74	47				
	3	10 ⁴	16	10	.0024		.29	
		2x10 ⁴	68	Tm				
	5	10 ⁴	0	5	.002		.25	
		2x10 ⁴	55	24				
	7	5x10 ⁴	61	81	.0014		.18	
		10 ⁵	Tm	Tm				
	9	5x10 ⁴	17	25	.0004		.05	
		10 ⁵	84?	Tm				
10 ⁻³	0	1000	2	0	.008 .0069	.0081 .0069	1	
		2000	7	9				
		5000	43	38				
	2	5000	4	3	.0058		.71	
		10 ⁴	60	55				
	3	10 ⁴	37	50	.0044		.54	
		2x10 ⁴	Tm	Tm				
	5	10 ⁴	10	21	.0026		.32	
		2x10 ⁴	63	62				
	7	5x10 ⁴	98	97	.002		.24	
		10 ⁵	Tm	Tm				
	9	5x10 ⁴	34	39	.0007		.09	
		10 ⁵	Tm	Tm				

EFFICIENCY LINE 22-206



[illegible]

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 22004

ARC USE ONLY

P.O. # _____
 ORDER DATE _____
 REF # _____
 CONTACT _____
 EST AMT _____
 SCHED DEL _____
 SPECIAL ROUTING _____
 NON COM VENDOR _____
 FLAGGED BY PROTOCOL _____

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASIS

REQUEST BY: Dr. Greg Sibley DATE: _____

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [Signature]

FAS ACCOUNT 6-9550-5100 PI: Hallahan

VENDOR: FCR1 PROTOCOL: 58671

REQUESTED DELIVERY DATE: _____ PHONE: 2-6819

SPECIES: mouse QUANTITY: 48

STRAIN: Atmy mic Nude SEX: M ☒ F EITHER

WEIGHT/AGE: 5-6 weeks ALTERNATE WEIGHT/AGE: _____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER ☒ CLSC _____ FMI _____ OTHER _____

V87 - TNF

EFFICIENCY LINE 22-206

Purpose - Quantify TNF production in V87 cell line
with 899-6 virus +/- 5 Gy RT.
Use 3616 virus as control

Design -

- Fixed variables - cell type V87

- intervals = infect x 2 hrs → RT

- aliquot media @ 0, 2, 4, 6 hrs then
subculture & save 10^6 cells

- Study variables - virus: None, 3616, 899-6 @ $MOI=10^{-1}$
RT: 0 Gy, 5 Gy

Conclusion - 10^5 PFU too little to show TNF production c.
899-6

METHODS -

- ① Plate V87 cells in 60 mm dishes.
Grow to subconfluent. Need ~~20~~²⁵ dishes
- ② Count 1 dish to determine # PFU needed for
MOI of 10^{-1} . No. Infect $\bar{c} = 10^5$ PFU (use
this also for other cell lines to compare TNF production
i.e. TNF production should be dependent only on PFU &
not on cell number (MOI not important)).

$$899,646 \text{ cells} = 1.2 \times 10^9 \text{ PFU/ml} : \frac{10^5 \text{ PFU}}{1.2 \times 10^9 \text{ PFU/ml}} = 0.83 \text{ ml of } 1.2 \times 10^5 \text{ d.i.} (+.17 \text{ media})$$

$$3616 \text{ cells} = 2.3 \times 10^9 \text{ PFU/ml} : \frac{10^5}{2.3 \times 10^9 \text{ PFU/ml}} = 0.43 \text{ ml " } 2.3 \times 10^5 \text{ " (+.57 media)}$$

- ③ Infect cells $\times 2^\circ$ in 1 ml 199K media.

Add

- ④ Remove inoculum & add 6 ml of 10% FCS media
(V87 media) + IgG.

- ⑤ Irradiate $\bar{c} 5 \text{ Gy}$

- ⑥ Aliquot 1 ml media @ $0, 2, 4, 6^\circ$ & subculture in 5n
~~@ 10^6 cells/ml~~ \rightarrow save ~~as~~ 1 ml as "pellet".

- ⑦ Centrifuge aliquots before using for TNF ELISA assay
- ⑧ To calculate total TNF:

$$899,646 = ([0^\circ] \times 1 \text{ ml}) + ([2^\circ] \times 1 \text{ ml}) + ([4^\circ] \times 1 \text{ ml}) + ([6^\circ] \times 3 \text{ ml}) + ([6^\circ \text{P}] \times 2 \text{ ml})$$

$$3616 = ([6^\circ] \times 6 \text{ ml}) + ([6^\circ \text{P}] \times 6 \text{ ml})$$

$$0 = ([6^\circ] \times 6 \text{ ml}) + ([6^\circ \text{P}] \times 2 \text{ ml})$$

V-87-R899-6

Purpose - Generate Cell Survival Curve for V-87 cells
with different Mol's of 899-6 virus.

Design - Fixed Variables - Cell Type V87
- intervals = infect x 2°
- Virus = 899-6

Study Variables = Mol = 2, 1, .5, 10^{-1} , 5×10^{-2} , 10^{-2} ,
 5×10^{-3} , 10^{-3} , 10^{-4}

V-87

EFFICIENCY LINE 22-206



METHODS -

① Plate 5×10^5 V87 cells / 60 mm plate, grow to sub-confluence.

② Subculture & count a plate = 2.4×10^6 cells
 Titer of 899-6 = 1.2×10^6 PFU/ml \rightarrow dilutions

Mol 2 = $4.8 \times 10^6 / 1.2 \times 10^7 = .4$ ml

Mol 1 = $2.4 \times 10^6 / 1.2 \times 10^7 = .2$ "

Mol 0.5 = $1.2 \times 10^6 / 1.2 \times 10^7 = .1$ "

Mol 0.1 = $2.4 \times 10^5 / 1.2 \times 10^6 = .2$ "

Mol 5×10^{-2} = $1.2 \times 10^5 / 1.2 \times 10^6 = .1$ "

Mol 10^{-2} = $2.4 \times 10^4 / 1.2 \times 10^5 = .2$ "

Mol 5×10^{-3} = $1.2 \times 10^4 / 1.2 \times 10^5 = .1$ "

Mol 10^{-3} = $2.4 \times 10^3 / 1.2 \times 10^4 = 0.2$ "

Mol 10^{-4} = $2.4 \times 10^2 / 1.2 \times 10^3 = .2$ "

③ Infect x 2 hrs

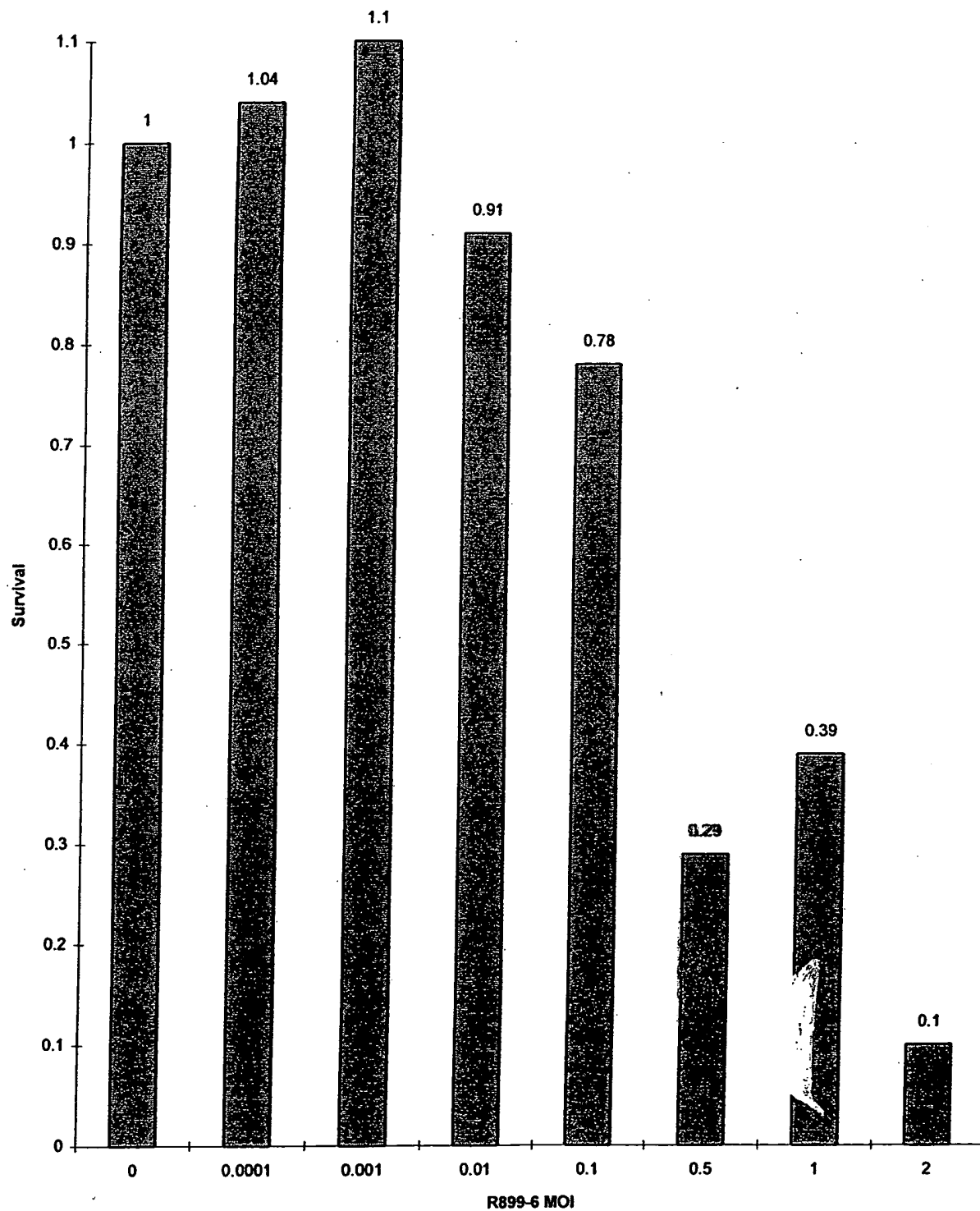
④ Subculture to 100 mm plates in 10% FCS media (+ IgG

⑤ Stain & count p ~ 3 wks (too soon = plaques too sm)

EFFICIENCY LINE 22-206

[illegible]

Survival By R899-6 MOI



U-87

CONCLUSIONS -

- ① R899-6 doesn't appear to have an appreciable effect until $MOI \sim 0.1$
- ② ? $TCD_{50} \sim MOI = 0.5$
- ③ Repeat using MOI 's of 0, 10^{-2} , 10^{-1} , 0.5, 1, 2, 3, 5.
- ④ Plating efficiency still very low (2%), try using conditioned media (50/50)

EFFICIENCY LINE # 22-206

TNF ELISA

Adeno - TNF Expression (#53)

2.2x10⁸ pfu/tu

	1	2	3 Adeno	4 Egr TNF	5 + single	6 S Gyr fraction	7	8	9	10
A ¹	0		1394 R		1406 R		1410 R		1394 L	
B ²	15.7		1396 R		1403 R		1405 R		1396 L	
C ³	31.3		1434 R		1404 R		1412 R		1434 L	
D ⁴	62.5		1397 R		1405 R		1413 R		1397 L	
E ⁵	125		1398 R		1402 R		1414 R		1398 L	
F ⁶	250		1399 R		1407 R		1411 R		1399 L	
G ⁷	500		1400 R		1408 R		1416 R		1400 L	
H ⁸	1000		1401 R		1409 R		RIPX BLK		1401 L	
		Adeno Ø			Adeno - Ø					
A ¹¹	1406 L		1410 L		1421		1417		1433 R	
B ²	1403 L		1405 L		1422		1418		1433 L	untreated tumor
C ³	1404 L		1412 L		1423		1420		1419	
D ⁴	1405 L		1413 L		1425		1424		med. BLK - Gyr	
E ⁵	1402 L		1414 L		1436 R		1427		1899-6 0°	
F ⁶	1407 L		1411 L		1436 L	untreated tumor	1429		2 " 0 Gyr	
G ⁷	1408 L		1416 L		1428		1426		3 899-6 2°	
H ⁸	1409 L		RIPX BLK		1435		1430		4 " 0 Gyr	
A ¹⁰	5 899-6 4°	22	22 899-6 4°	24 20	25	26	27	28		
B ²¹	6 " 0 Gyr		13 None	5 Gyr	21 None 6°		29 36 16 6°			
C ²²	7 899-6 6°		14 " 5 Gyr		22 " 0 Gyr		30 " 0 Gyr			
D ³	8 " 0 Gyr		15 899-6 4°		23 None P		31 36 16 P			
E ²⁴	9 899-6 P		16 " 5 Gyr		24 " 0 Gyr		32 " 0 Gyr			
F ²⁵	10 " 0 Gyr		17 899-6 6°		25 None 6°		33 36 16 6°			
G ²⁶	11 899-6 0°		18 " 5 Gyr		26 " 5 Gyr		34 " 5 Gyr			
H ²⁷	12 " 5 Gyr		19 899-6 P		27 None P		35 36 16 0 P			
			20 " 5 Gyr		28 " 5 Gyr		36 " 5 Gyr			

EFFICIENCY LINE 22-206

[TNF] By Virus Type (10 exp5 PFU) and RT Dose in U-87 Cells

Virus	Dose	Time	[TNF]	Total [TNF]
899-6	0	0	0.19	62.52
899-6	0	2	2.34	
899-6	0	4	4.99	
899-6	0	6	11.04	
899-6	0	p	10.94	
899-6	5	0	0.94	64.37
899-6	5	2	1.49	
899-6	5	4	4.99	
899-6	5	6	12.29	
899-6	5	p	10.04	
None	0	6	6.64	42.62
None	0	p	1.39	
None	5	6	3.64	24.42
None	5	p	1.29	
R3616	0	6	5.44	42.92
R3616	0	p	5.14	
R3616	5	6	11.09	
R3616	5	p	5.34	

MOLECULAR DEVICES

Kinetic KINETICS MICROPLATE READER

PLATE #: 3



OPERATOR: _____

DATE: _____

O.D. LIMIT: 3.000

TIME: _____ AM/PM

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: ON

NOTES: _____

CAL: ON

OPTICAL DENSITY

	25	26	27	28	29	30	31	32	33	34	35	36
A	0.063	0.176	0.073	0.066	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.081	0.070	0.095	0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.067	0.076	0.080	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.068	0.074	0.090	0.113	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.073	0.079	0.095	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.101	0.077	0.106	0.158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.076	0.088	0.095	0.094	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.063	0.070	0.031	0.083	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000

$$(y - .0643) / .005$$

$$y = .005x + .0643$$

$$y = .0657 e^{.6004x}$$

$$\ln y - \ln .0657 = .6004x$$

MOLECULAR DEVICES

Vmax KINETICS MICROPLATE READER

PLATE #: 1

ID#

V87

DATE: _____

OPERATOR: Greg

O.D. LIMIT: 2.000

TIME: _____

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: ON

NOTES: _____

CAL: 0

Standards

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.086	0.097	0.101	0.092	0.093	0.080	0.073	0.090	0.072	0.127	0.101
B	0.129	0.144	0.070	0.072	0.375	0.366	0.272	0.236	*	0.077	0.091	
C	0.199	0.198			0.732	0.316	0.490	0.622	1.411	1.002	0.366	0.313
D	0.371	0.367	0.343	0.142	0.068	0.068	0.161	0.149	1.978	1.448	0.083	0.170
E	0.670	0.727	0.154	0.153	0.237	0.238			1.205	1.131	0.127	0.140
F	1.333	1.365	0.680	0.660	0.647	0.635	0.148	0.110	2.623	2.650	0.198	0.166
G	2.607	2.460			1.774	1.277	0.125	0.085	0.726	0.700	0.121	0.116
H			0.203	0.143	2.161	2.483	0.088	0.063	0.131	0.099	0.600	0.627

MOLECULAR DEVICES

Vmax KINETICS MICROPLATE READER

PLATE #: 1

ID#

DATE: _____

OPERATOR: _____

O.D. LIMIT: 2.000

TIME: _____

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: ON

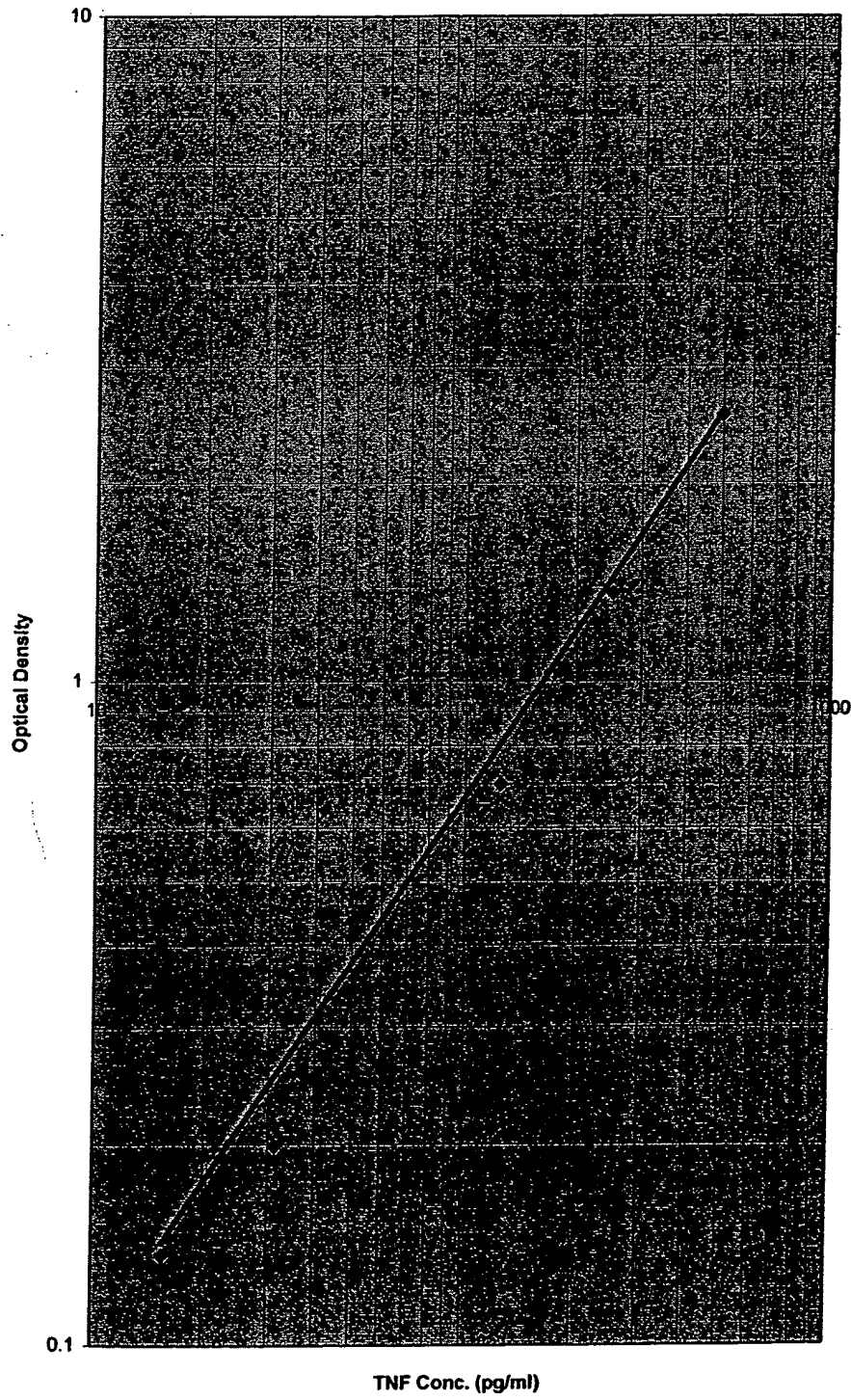
NOTES: _____

CAL: 0

OPTICAL DENSITY

	13	14	15	16	17	18	19	20	21	22	23	24
A	0.075	0.070	0.109	0.180	0.327	0.329	0.141	0.122	0.097	0.087	0.077	0.070
B	0.225	0.267	0.146	0.180	0.137	0.131	0.108	0.105	0.090	0.083	0.072	0.068
C	0.810	0.841	0.175	0.609	0.074	0.075	0.219	0.212	0.127	0.117	0.088	0.082
D	0.250	0.263	0.340	0.255	0.123	0.125	0.059	0.059	0.111	0.120	0.056	0.071
E	0.583	0.716	0.079	0.082	0.083	0.097	0.055	0.060	0.109	0.122	0.120	0.125
F	0.126	0.134	0.123	0.185	0.046	0.048	0.067	0.070	0.124	0.121	0.130	0.126
G	0.271	0.242	0.058	0.102	0.031	0.032	0.063	0.071	0.057	0.071	0.109	0.119

TNF ELISA Plot



V87
R899-6

EFFICIENCY LINE # 22-206

PURPOSE - Generate cell survival curve for V-87 cells
- with different mol's of 899-6 virus

Design - Fixed variables - cell type V87
- intervals = infect x 2°
- virus 899-6

Study variables - mol: $0, 10^{-2}, 10^{-1}, 0.5,$
 $1, 2, 3, 5$

V87

EFFICIENCY LINE 22-206

METHODS -

① Grow V87 cells to subconfluence on 60 mm plates
 10^6 plated.

② Subculture & count a plate = 2×10^6
 899-6 titer = 1.2×10^9 PFU/ml (from titer)

MOI 5 = $\frac{1 \times 10^7}{1.2 \times 10^9} = .83$ ml

MOI 3 = $\frac{6 \times 10^6}{1.2 \times 10^9} = .5$

MOI 2 = $\frac{4 \times 10^6}{1.2 \times 10^9} = .33$

MOI 1 = $\frac{2 \times 10^6}{1.2 \times 10^9} = .17$

MOI 0.5 = $\frac{1 \times 10^6}{1.2 \times 10^9} = .083, 83$

MOI 0.1 = $\frac{2 \times 10^5}{1.2 \times 10^9} = .17$

MOI 10^{-2} = $\frac{2 \times 10^4}{1.2 \times 10^9} = .17$

③ Infect $\times 2^\circ$ in 1 ml 199 V

④ Subculture to 100mm plates \bar{c} — ml Conditioned media per plate

⑤ Stain & count $\bar{p} \sim 3$ wks (18-21 d)

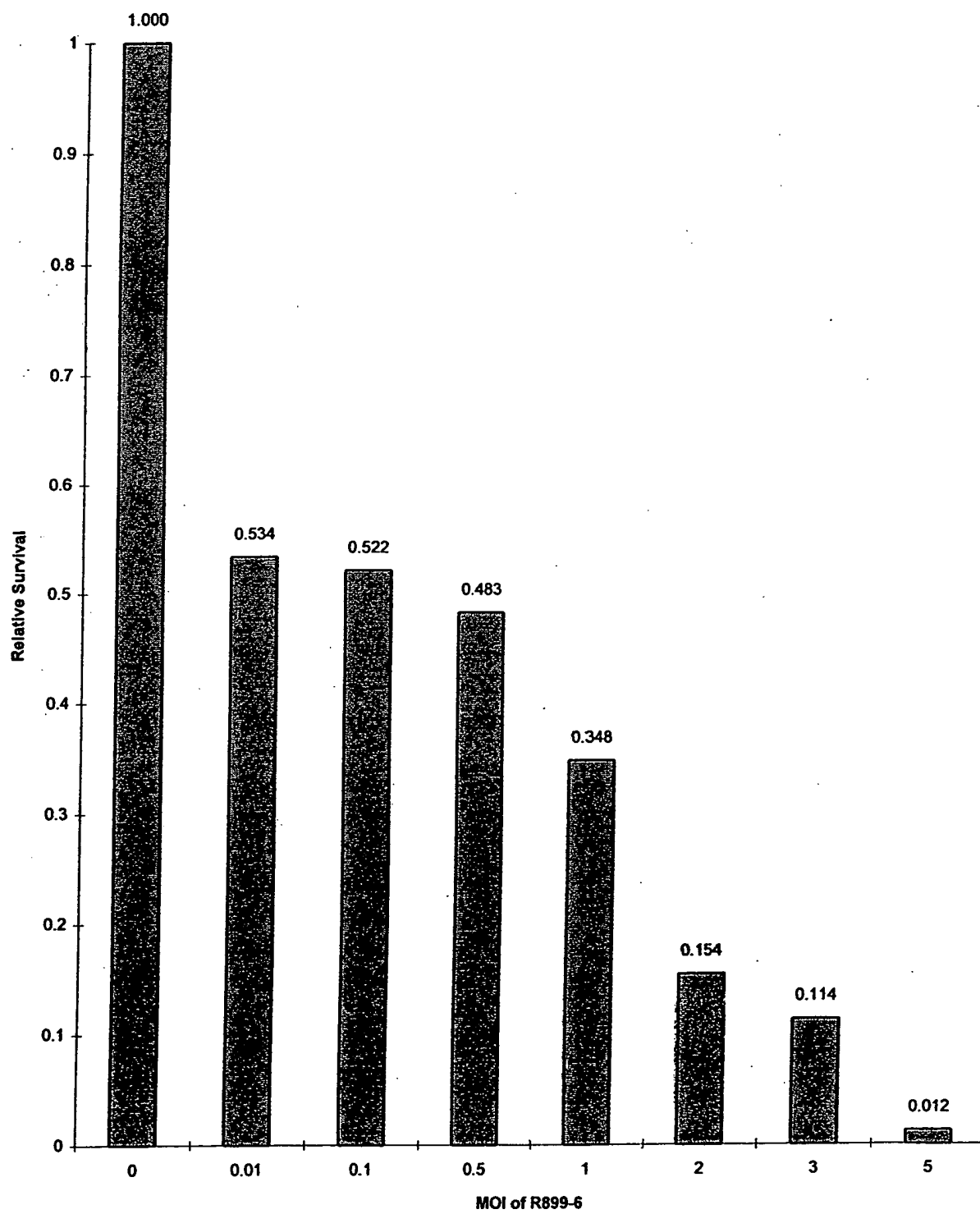
EFFICIENCY LINE 22-206

[illegible]

Survival of U-87 Cells by MOI of R899-6

MOI	# plated	# counted			Ave. Surv.	P.E.	Rel. Surv.
0	2000	21	12	19	0.0107	0.0107	1
	5000	68	64	59			
0.01	2000	5	6	6	0.005717	0.0107	0.534268
	5000	53	28	48			
0.1	2000	4	11	6	0.005583	0.0107	0.521807
	5000	30	50	35			
0.5	5000	34	22	21	0.005167	0.0107	0.482866
	10000	51	70	35			
1	10000	36	37	24	0.003725	0.0107	0.348131
	20000	90	83	80			
2	10000	18	13	17	0.00165	0.0107	0.154206
	20000	33	25	44			
3	20000	18	17	14	0.001215	0.0107	0.113551
	50000	69	84	89			
5	50000	7	8	5	0.000133	0.0107	0.012461
MOI	Rel. Surv.						
0	1.000						
0.01	0.534						
0.1	0.522						
0.5	0.483						
1	0.348						
2	0.154						
3	0.114						
5	0.012						

Relative Survival By R899-6 MOI in U-87 Cells



V-87 Mycoplasma assay

	1	2	3	4	5	6	7	8	9
1									
2	PURPOSE - Rule out mycoplasma infection as reason								
3	for poor plating efficiency i V87 cells								
4									
5									
6	METHODS - see attached protocol								
7									
8									
9	CONCLUSION - No mycoplasma infection								
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

U-87 - TNF

Purpose - Quantify TNF production in U87 cell line
 c R3616, 899-6 & Ø virus c 0 or 9 Gy
 (not of virus - 0.5) Use 2×10^6 PFU ($\mu\text{mol} = 1$)

METHODS -

- ① Plate U87 cells in 60 mm dishes & grow to subconfluence
- ② 899-6: 2×10^6 PFU / 1.2×10^8 PFU/ml = .17 ml
 3616: 2×10^6 PFU / 2.3×10^8 PFU/ml = .87 ml
- ③ Infect x 2° in total volume 1 cc 199V media
- ③ Remove inoculum & add 6 ml of 10% FCS media
- ~~④ Aliquot 1 ml media @ 2, 6~~
- ④ Inactivate c 9 Gy RT 11 am 3 pm 9 pm 9 am 3 pm
- ⑤ Aliquot 1 ml media @ 2°, 6°, 12°, 24°, 30°
 then subculture in 3 ml and save 1 ml as pellet

EFFICIENCY LINE 22-206



1	2	3	4	5	6	7	8	9	
<u>RESULTS -</u>									
<u>VIRUS</u>	<u>RT</u>	<u>Time</u>	<u>[TNF]</u>	<u>total</u> <u>[TNF]</u>	<u>VIRUS</u>	<u>RT</u>	<u>Time</u>	<u>[TNF]</u>	<u>total</u> <u>[TNF]</u>
0	0	0°			3616	0	0°		
		6°					6°		
		12°					12°		
		24°					24°		
		30°					30°		
		P					P		
0	96y	0°			3616	96y	0°		
		6°					6°		
		12°					12°		
		24°					24°		
		30°					30°		
		P					P		
899-6	0	0°							
		6°							
		12°							
		24°							
		30°							
		P							
899-6	96y	0°							
		6°							
		12°							
		24°							
		30°							
		P							

TNF ELISA Assay: U-87 Cells Infected with R899-6, R3616, or No Virus (MOI=0.8) +/- 9 Gy RT

Standards

reading 1	reading 2	[TNF]	ave. reading
0.087	0.087	15.6	0.087
0.119	0.137	31.3	0.126
0.205	0.217	62.5	0.211
0.39	0.407	125	0.399
0.733	0.756	250	0.745
1.354	1.459	500	1.407
1.997	2.284	1000	2.141

				Virus	Dose	Time	Total [TNF]
0.044	0.044	7.8	0.044	None	0	0	62.60
0.045	0.046	8.1	0.046	None	0	10	
0.04	0.046	7.6	0.043	None	0	21	
0.044	0.042	7.6	0.043	None	0	27	
0.043	0.047	8.0	0.045	None	0	P	
0.04	0.048	7.8	0.044	None	9	0	67.73
0.051	0.046	8.8	0.049	None	9	10	
0.052	0.054	9.8	0.053	None	9	21	
0.051	0.048	9.0	0.050	None	9	27	
0.043	0.045	7.8	0.044	None	9	P	
0.047	0.041	7.8	0.044	R3616	0	0	72.24
0.047	0.044	8.1	0.046	R3616	0	10	
0.042	0.055	8.8	0.049	R3616	0	21	
0.054	0.059	10.6	0.057	R3616	0	27	
0.05	0.047	8.8	0.049	R3616	0	P	
0.046	0.042	7.8	0.044	R3616	9	0	67.48
0.046	0.04	7.6	0.043	R3616	9	10	
0.051	0.05	9.2	0.051	R3616	9	21	
0.046	0.048	8.4	0.047	R3616	9	27	
0.048	0.048	8.7	0.048	R3616	9	P	
0.054	0.05	9.6	0.052	R899-6	0	0	3004.23
1.476	1.587	633.2	1.532	R899-6	0	10	
1.836	1.48	698.6	1.658	R899-6	0	21	
1.752	1.973	806.9	1.863	R899-6	0	27	
0.08	0.08	16.3	0.080	R899-6	0	P	
0.047	0.051	8.9	0.049	R899-6	9	0	3323.99
0.939	0.829	320.5	0.884	R899-6	9	6	
1.503	1.569	635.5	1.536	R899-6	9	10	
1.83	1.96	824.4	1.895	R899-6	9	21	
2.076	1.99	899.4	2.033	R899-6	9	27	
0.09	0.089	18.8	0.090	R899-6	9	P	

V-87 ~~7.1.1~~
R3616

EFFICIENCY LINE 22-206



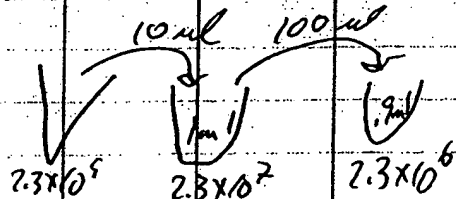
1 Purpose - Survival of V82 cells \bar{c} ~~R3616~~ ^{R3616} @ mol of
2 0 (control) & 0.5 \bar{c} RT doses :: 0, 3, 5, 7, 9 Gy

5 METHODS -

- 6 ① Plate 10^6 cells in 60 mm dish (12/9) & grow to
- 7 confluence
- 8 ② Cant a dish & infect ~~one~~ one dish \bar{c} mol 0.5 of
- 9 ~~R3616~~ R3616
- 10 ③ Infect x 2° in 1 ml 199V @ 37°
- 11 ④ Subculture into 100 mm plates \bar{c} 50% Cond. Media
- 12 & (+ IgG). Wait 6.
- 13 ⑤ Irradiate @ 0, 3, 5, 7, 9 Gy
- 14 ⑥ Incubate x 3 wks
- 15 ⑦ Stain & Count
- 16 ⑧ Adjust media ~~at 36~~ @ 1 wk for [TNP]
- 17 (? is there induction \bar{c} 7 doses RT

20 Dish cant = 2.3×10^6 cells

22 R3616 titer = 2.3×10^9 AFU/ml



28 Use 0.5 cc 2.3×10^6 dilution = mol 0.5

EFFICIENCY LINE 22-206

[illegible]

V-87
R899-6

EFFICIENCY LINE 22-206



Purpose - Generate cell survival curve for V-87 cells
with RT doses 0, 5, 9 Gy & MOI's of 899-6 =
~~0.5~~, ~~1.5~~, 0

METHODS ① Plate 10^6 cells per 60 mm dish = V-87

② When subconfluent (2 days) infect \bar{c} virus in
1 cc 199V x 2° .

MOI Titer R899-6 = 1.2×10^9 pfu/ml
MOI = 1 = ~~1.6×10^6~~ / 1.2×10^7 = 0.133

\bar{c} virus in
5 ml in
V $\frac{0.45}{1.2 \times 10^9}$ $\frac{1.41}{1.2 \times 10^7}$ $\frac{1.45}{1.2 \times 10^6}$

MOI = 0.5 = $\frac{0.8 \times 10^6}{1.2 \times 10^6}$ = 0.667

~~MOI = 0.1 = $\frac{1.6 \times 10^5}{1.2 \times 10^6}$ = 0.133~~

③ Subculture into 10 mm dishes at appropriate concentrations
in 50% conditioned media.

④ Let cells adhere x 6°

⑤ Irradiate

⑥ Incubate x 3 wks, stain & count.

EFFICIENCY LINE# 22-206

[illegible]

Purpose - Assess toxicity of R999-6 & R3616 in
rod mice

METHODS -

② 8 mice

R899-6: 10 μ l of 1.2×10^9 PFU/ml = 1.2×10^7 PFU (2 mice)

10 μ l of 1.2×10^8 PFU/ml = 1.2×10^6 PFU (2 mice)

R3616: 10 μ l of 2.3×10^9 PFU/ml = 2.3×10^7 PFU (2 mice)

10 μ l of 2.3×10^8 PFU/ μ l = 2.3×10^6 PFU (2 mice)

Cage AA104551 -	{ 1681	Ø	R888-6	1.2 x 10	7
	{ 1682	L	"	"	
	{ 1683	R	"	1.2 x 10	6
	{ 1684	LR	"	"	

Cage AA104552-	1685	Ø	23616	2.3x10 ⁷
	1686	L	"	"
	1687	R	"	2.3x10 ⁶
	1688	LR	"	"

(2) Injected 3⁰⁰ PM 1/5/94

RESULTS -

No local or systemic effects noted
Mice examined twice weekly for weeks
Soc'd on



Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

As per our telephone conversation on please place the following mice on TMP-
SMZ (Bactrim) for 1 week:

Cages AA105283, AA104542 through AA104545, AA104547 through AA104550,
and AA107678 through AA107710.

This should include **all** mice from Hallahan's Lab (Radiation Oncology) currently in
Cummings Room #1053.

Thank you. If you have any questions you can contact me at beeper #3439 or contact
Helena at 2-0294.

Sincerely,

Gregory S. Sibley, M.D.

REQ NO 18777

ARC USE ONLY

P.O. # _____
 ORDER DATE _____
 REF. # _____
 CONTACT _____
 EST AMT _____
 SCHED DEL _____
 SPECIAL ROUTING _____
 NON COM VENDOR _____
 FLAGGED BY PROTOCOL _____

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASIS

REQUEST BY: Gregory Sibbey MD DATE: _____

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: Mohamed R. Sibbey

2-73731-5100

FAS ACCOUNT: FCR1

VENDOR: FCR1

REQUESTED DELIVERY DATE: _____

PI: Hq/Hagan

PROTOCOL: 58621

PHONE: 2-6819 2-0294

SPECIES: Mouse QUANTITY: 60

STRAIN: Polymic Nude SEX: M ☒ F ☐ EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: _____
 (IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
 (RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER ☒ CLSC _____ FMI _____ OTHER _____

V87 TNF (intervals)

Purpose - To determine optimal interval from infect (R899-6) to RT (9 Gy) to optimize TNF production in V87 cells

Methods

① Plate 10^6 V87 cells per 60 mm dishes. Incubate $\sim 36^\circ$ for $\sim 2 \times 10^6$ cells

② Infect plates with 10^6 PFU of R899-6 at
 12° prior to RT = 10^{pm}
 8° prior to RT = 2^{pm}
 4° " " " = 6^{pm} (Actually $6^{15} = 3.25$ hrs)
 2° " " " = 8^{pm}

Titer R899-6 = 1.2×10^9 PFU/ml

\therefore Use 0.83 ml of 1.2×10^6 PFU/ml dilution + 199U media to total vol = 1.5 cc

③ Add 2.5 ml 10^6 FCS media + IgG (total vol 4 cc)
 "Control" dishes \rightarrow Aliquot 1 ml as "supernatant"
 For Decant remaining media
 Trypsinize cells \pm 1 ml trypsin + 1 ml Verbe
 then add 2 ml media (total vol = 4)
 Aliquot 1 ml as "pellet"

④ For "treatment" dishes RT \pm 9 Gy
 Wait 7° then repeat aliquot procedure for
 Supernatant & pellet as above

TNF ELISA

U-87 Cells

reading 1	reading 2	average	[TNF]	Time	Sample	Average	Total [TNF]	Rel. [TNF]
1.267	1.308	1.2875	325.978	12 hours	control-super	1283.22	1623.47	1239.04
1.248	1.256	1.252	315.632	12 hours	control-super			
0.38	0.353	0.3665	76.5162	12 hours	control-pellet	340.25		
0.474	0.399	0.4365	93.6086	12 hours	control-pellet			
2.331	2.31	2.3205	643.123	12 hours	9 Gy-super	2394.87	2862.506	
2.053	2.027	2.04	554.311	12 hours	9 Gy-super			
0.505	0.534	0.5195	114.425	12 hours	9 Gy-pellet	467.638		
0.537	0.541	0.539	119.394	12 hours	9 Gy-pellet			
0.417	0.422	0.4195	89.416	8 hours	control-super	360.864	642.5502	2071.4
0.421	0.431	0.426	91.016	8 hours	control-super			
0.311	0.34	0.3255	66.73	8 hours	control-pellet	281.686		
0.343	0.37	0.3565	74.113	8 hours	control-pellet			
1.455	1.503	1.479	382.519	8 hours	9 Gy-super	1953.96	2713.952	
2.127	2.208	2.1675	594.462	8 hours	9 Gy-super			
0.587	0.607	0.597	134.332	8 hours	9 Gy-pellet	759.989		
0.957	1.058	1.0075	245.662	8 hours	9 Gy-pellet			
0.065	0.058	0.0615	9.76247	3.25 hours	control-super	40.8921	130.1639	1371.14
0.065	0.068	0.0665	10.6836	3.25 hours	control-super			
0.085	0.087	0.086	14.3726	3.25 hours	control-pellet	89.2718		
0.158	0.17	0.164	30.2632	3.25 hours	control-pellet			
0.933	0.991	0.962	232.909	3.25 hours	9 Gy-super	906.04	1501.304	
0.901	0.931	0.916	220.111	3.25 hours	9 Gy-super			
0.709	0.714	0.7115	164.467	3.25 hours	9 Gy-pellet	595.264		
0.599	0.586	0.5925	133.165	3.25 hours	9 Gy-pellet			
0.059	0.068	0.0635	10.1296	2 hours	control-super	41.0714	131.0586	2360.16
0.065	0.065	0.065	10.4061	2 hours	control-super			
0.067	0.112	0.0895	15.0494	2 hours	control-pellet	89.9873		
0.179	0.146	0.1625	29.9442	2 hours	control-pellet			
1.414	1.524	1.469	379.538	2 hours	9 Gy-super	1679.41	2491.216	
1.687	1.785	1.736	460.167	2 hours	9 Gy-super			
0.859	0.824	0.8415	199.593	2 hours	9 Gy-pellet	811.806		
0.866	0.866	0.866	206.311	2 hours	9 Gy-pellet			

V-87 TNF (intervals)

PURPOSE - To determine optimal interval from infection (R899-6) to RT (9 Gy) to optimize ~~the~~ subsequent TNF induction experiments

METHODS -

① Plate 10^6 V87 cells per 60 mm dish. Incubate $48 \sim 36^\circ$ for $\sim 2 \times 10^6$ cells

② Infect plates \bar{c} 10^6 PFU R899-6
= 0.83 ml of 1.2×10^6 PFU/ml dilution
+ 199V media to \bar{c} 1.5 ml

Infect @ 7^{30}_{AM}

③ Aliquot & trypsinize "control dishes" @
 4° \bar{p} infection 11^{30}
 6° " " 1^{30}
 8° " " 3^{30}

④ Irradiate @ same time points \bar{c} 9 Gy

⑤ Aliquot & trypsinize "treatment dishes" @
 7° \bar{p} RT = 6^{30}_{PM} , 8^{30}_{PM} , 10^{30}_{PM}

IN VIVO - TNF induction - intervals U87 hind limbs nude mice

PURPOSE - To determine optimal interval from infection → RT
in U87 tumors in hind limbs of nude mice for TNF
production

METHODS -

① Transplant 1mm^3 U87 tumors into hind limbs of
nude mice. Grow to $\sim 6\text{mm}$ (range 4.5-8mm)
greatest dimension.

② Infect mice in groups of 4 @ 48° , 24° , 10° & 5°
prior to RT.

- $48^\circ = 4\text{PM } 1/24$

- $24^\circ = 4\text{PM } 1/25$

- $10^\circ = 7\text{AM } 1/26$

- $5^\circ = 12\text{N } 1/26$

$0^\circ = 5\text{PM } 1/26 = \text{Irradiate} = 20\text{Gy single fraction.}$

③ Sacrifice mice @ 4PM

Greg's Mouse Log

Mouse #	Cage #	DOB	Tumor Date	Infect Date	RT Date	Date Sacrif	Tumor size
1703	AA104542			R			7.5 8x5x3.5
1711	AA104542			Ø			6 5.5x5x2.5
1712	AA104542			L			8 10x8.5x6
1714	AA104542			LR			6 6x6x3.5
1701	AA104543			Ø			6.5 7.5x6x4
1715	AA104543			Ø → L			4.5 5.5x5x3
1718	AA104543			LR			5.5 4x5.5x2.5
1721	AA104543			Ø → R			7 6x7.5x3
1716	AA104544						3.5
1722	AA104544						3.5
1723	AA104544						3
1724	AA104544						2
1689	AA104546						
1690	AA104546						
1691	AA104546						
1692	AA104546						
1693	AA104547			Ø			10
1694	AA104547						20
1695	AA104547			SqC			0
1696	AA104548			LR			6 6.5x5.5x3.5
1698	AA104548			L			4 9x9.5x8
1699	AA104548			R			5 6x5x3
1700	AA104548			LR → LRR			6 4.5x2.5x2
1697	AA104549						3.5
1702	AA104549						2
1713	AA104549						4.5
1717	AA104549						2.5
1704	AA104550						3.5
1705	AA104550						
1706	AA104550			SqC			0
1719	AA104550						3.5
1720	AA104550						3.5
1681	AA104551						
1682	AA104551						
1683	AA104551						
1684	AA104551						
1685	AA104552						
1686	AA104552						
1687	AA104552						
1688	AA104552						
1707	AA105283						0
1708	AA105283						3
1709	AA105283						2
1710	AA105283						3

34°
80°

48°

5

$$\frac{4}{3} \pi r^3$$

$$\frac{4}{3} \pi \left(\frac{d}{2}\right)^3$$

$$\frac{4}{3} \pi \frac{d^3}{8}$$

$$\frac{d^3}{2}$$

1/2 5170 wt

1867	23	20.5	12	25.1
1868	23	24	20.5	22.3
1869	21	23	15	24.2
1870	20	18	13	26.9

4550 Control

1719	20	14.5	10	21.8
1722	21	23	19	25.9
1717	17	15.5	12	23.7

5283 891-b

1710	12.5	1.5	3	20.7
1713	0	0	0	22.0
1720	11.5	1.5	1.5	26.0

4549 3016

1617	14.5	12	2.5	26.3
1723	0	0	0	27.1
1709	5.5	6	1.5	25.8
1708	3	3.5	2	21.0

8908 3016

1875	17.5	1.5	7	22.3
1876	14	10.5	10	24.9
1877	1.5	1.5	11	25.5
1878	1.5	1.5	10	20.0

8972 RT

1859	9.5	10	0	22.8
1860	13	11.5	6	19.0
1861	10	12	5	17.1
1862	9	9	3.5	23.3

8964 RT

1891	7	7	5.5	19.0
1892	12.5	12.5	7	23.1
1893	10.5	1.5	7	22.4
1894	9	5.5	5	21.4

8909 879-6+RT

1871	7	7	5	23.7
1872	6	6	4	22.2
1873	5	2.5	5.5	20.2
1874	8.5	1.5	7	26.0

8967 894-6+RT

1877	0	0	0	24.5
1880	3.5	3	1	20.2
1881	5	6.5	2	23.9
1882	6.5	3.5	3.5	21.7

8971 3616+RT

1863	9.5	8	3.5	19.7
1864	11	7	3	23.2
1865	7	5.5	3.5	24.4
1866	7	5.5	4	25.3

8960 3616+RT

1883	4.5	7.5	2.5	20.7
1884	8.5	10	5	21.0
1885	12	8.5	6	19.0
1886	4	4	2	17.5

8963 10'36'10 wt

1875	13.5	12	7	20.6	5.3	12.5	7.5
1876	12	10.5	8	21.3	13.5	14	10
1877	10	"	7	23.7	10.5	8.5	7.5
1878	10	8	6	24.3	11	9	2.5

8965 899-6 all mice damaged 2228

1887	9	10.5	6	23.9
1888	10	7	5.5	24.0
1889	4	3.5	1	23.9
1890	6	6	4.5	22.4

8967 899-6 RT

1879	4	5	3	24.6	0	0	0
1880	3	3	2	19.5	0	0	0
1881	7	6	4	22.7	7	6	4
1882	7	6	4	20.0	6.5	4.5	3

8969 899-6 RT

1871	8	8	5	20.7	7	6	4.5
1872	8	4.5	4.5	20.6	8	5	4
1873	8.5	7	5.5	20.1	5	7.5	6
1874	10	8.5	5	23.6	8	7	4

8966 36'16 RT

1883	5	4	3.5	20.6	4	3.5	3.5
1884	10	11	7.5	21.1	11	15	6
1885	11	10	7.5	23.2	10.5	7	2.5
1886	5	6	3.5	19.9	4	3.5	2.5

8964 RT

1891	7	7.5	6	20.1	7	7	5
1892	9.5	7	7	24.0	7	7.5	6.5
1893	9	10	7.5	22.5	11	4.5	7
1894	9	9	6	21.8	9	9	6

Greg's mouse Log

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif	Day 0	weight	Day 3	weight
1704	DD104550	Control		None	None		9.5 8 6	228	12 14 9	698.63 24.8
1717	AA104550	Control		None	None		6 6.5 5.5 107.3		9.5 8 7	266 25.2
1719	AA104550	Control		None	None		7 7 6 147		8 8.5 5	170 21.4
1722	AA104550	Control		None	None		7 6.5 6.5 147.9		9.5 8 4	152 24.5
1867	AA108970	Control		None	None		8 6 5 120	23.7	10 8 6	240
1868	AA108970	Control		None	None		8.5 7 6.5 193.4	21.2	10 9.5 7.5	356.25
1869	AA108970	Control		None	None		8.5 7 4.5 133.9	22.7	10 9 7	315
1870	AA108970	Control		None	None		9 7 6 189	22.7	11 8.5 8	357
		Control					Mean: 158.3			319.36
							Std Error: 14.6			60.666
							Day: 0		Day: 3	3
1720	AA105283	10^7 899-6			None		Day 0		Day 3	
1710	AA105283	10^7 899-6			None		9 9 5.5 222.8	25	12 12 6.5	468
1707	AA105283	10^7 899-6			None		9 8.5 5.5 210.4	21.7	10 10 9	450
1713	AA105283	10^7 899-6			None		11 11 5 275.6	20	11 13 9	614.25
1887	AA108965	10^7 899-6			None		9 9 5 202.5	23	9 10 6.5	292.5
1888	AA108965	10^7 899-6			None		8.5 6 5 127.5	22.5	9.5 9 5	213.75 22.6
1889	AA108965	10^7 899-6			None		8.5 5.5 4.5 105.2	23.4	9 8 4.5	162 23.2
1890	AA108965	10^7 899-6			None		5.5 8 5 110	22.8	7 5.5 2.5	48.125 23.1
		10^7 899-6			None		7.5 6.5 5 121.9	23.3	8.8 8 5	176 22.9
							Mean: 172		Mean:	303.08
							Std Error: 22.57		Std Error:	67.448
							Day: 0		Day:	3
1697	AA104549	10^7 3616			None		Day 0		Day 3	
1708	AA104549	10^7 3616			None		9.5 7.5 7 249.4		11 11 7	423.5
1709	AA104549	10^7 3616			None		7.5 7 5 131.3		9.5 9 6	256.5
1723	AA104549	10^7 3616			None		7 8.5 4.5 133.9		9.5 10 6.5	308.75
1875	AA108968	10^7 3616			None		7 5.5 5 96.25		8.5 8 5.5	187
1876	AA108968	10^7 3616			None		11 10 7.5 412.5	20.1	13 12 8	598
1877	AA108968	10^7 3616			None		7.5 8 6 180	24.2	10 9 7.5	337.5
1878	AA108968	10^7 3616			None		9 7.5 6.5 219.4	24.2	10 9.5 8	380
		10^7 3616			None		6.5 8.5 4.5 124.3	24.8	8.5 8 5.5	187
							Mean: 193.4		Mean:	334.78
							Std Error: 36.26		Std Error:	48.124
							Day: 0		Day:	3

8971 3613 +RT wt.

1863	11	9	6.5	21.1	10.5	9.5	4.5
1864	11	7	5	21.4	10.5	8	9.5
1865	8	7.5	7.5	22.1	7.5	6.5	5
1866	7	8.5	5	22.7	7	6.5	5

8972 RT wt.

1859	11	11.5	7	23.3	10	10.5	5
1860	11.5	12	8.5	18.6	11.5	12	2.5
1861	12	11	7.5	20.1	10	9.5	6.5
1862	10	8.5	5	23.1	10	8	5

4549 3616 wt.

1697	12	9	5.5	25.6	12.5	11	6
1709	8	8	5	27.2	7	7	4
1723	6	6	3.5	25.6	5	5	2.5
1708	5	4	3.5	23.0	3	4	2.5

5083 819-6

1727	died	2/24	wt.				
1727	11.5	1.5	7.5	26.9	14	11	8
1713	0	0	0	21.7	0	0	0
1710	13.5	17	5.5	21.3	15	25	5

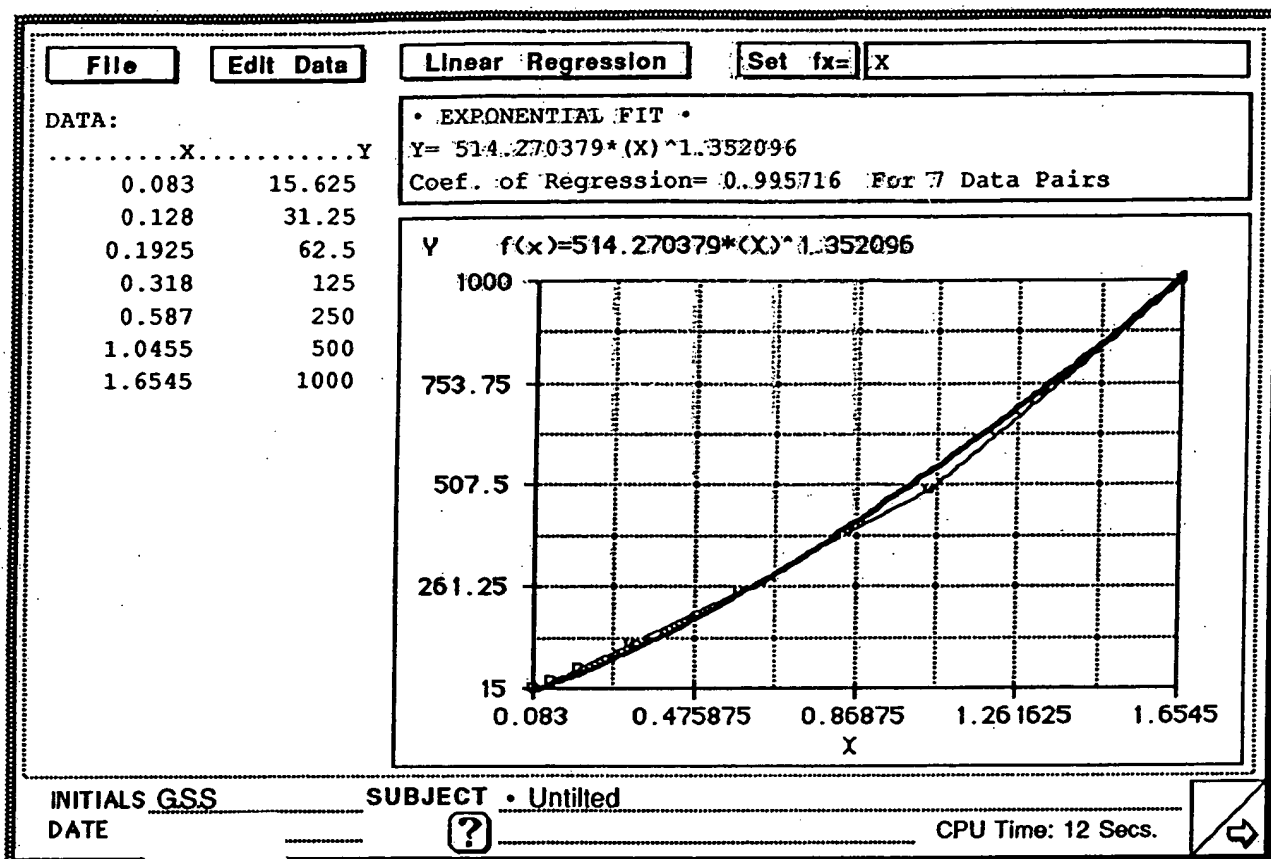
8970 control wt.

1867	18	19	12	23.9	20	16	10.5
1868	21	17.5	15.5	21.6	20.5	9.5	18.5
1869	18	16	11	25.5	21	12.5	12
1870	17	15	11	24.6	17.5	16.5	12

4550 control wt.

1719	11.5	14	9	22.0	17.5	13	12
1722	21	20	16.5	25.4	23	22.5	16.5
1717	15	18	18	24.7	17.5	16.5	13.5

[illegible]



TNF ELISA

U-87 Cells

reading 1	reading 2	average	[TNF]/ml	Time	Sample	[TNF]	Total [TNF]	Rel. [TNF]
0.053	0.063	0.058	10.9483	4 hours	control-super	41.0337	131.644	1497.08
0.053	0.052	0.0525	9.56856	4 hours	control-super			
0.096	0.114	0.105	24.4253	4 hours	control-pellet	90.6103		
0.089	0.098	0.0935	20.8799	4 hours	control-pellet			
0.717	0.721	0.719	329.222	4 hours	9 Gy-super	1275.75	1628.722	
0.646	0.725	0.6855	308.655	4 hours	9 Gy-super			
0.281	0.271	0.276	90.2195	4 hours	9 Gy-pellet	352.969		
0.281	0.253	0.267	86.265	4 hours	9 Gy-pellet			
0.111	0.13	0.1205	29.4229	6 hours	control-super	103.653	305.0815	1957.19
0.09	0.107	0.0985	22.4036	6 hours	control-super			
0.179	0.195	0.187	53.2992	6 hours	control-pellet	201.429		
0.177	0.166	0.1715	47.4151	6 hours	control-pellet			
0.875	0.872	0.8735	428.33	6 hours	9 Gy-super	1776.22	2262.27	
0.889	0.952	0.9205	459.781	6 hours	9 Gy-super			
0.344	0.344	0.344	121.512	6 hours	9 Gy-pellet	486.047		
0.36	0.328	0.344	121.512	6 hours	9 Gy-pellet			
0.305	0.332	0.3185	109.495	8 hours	control-super	387.223	660.3809	1933.52
0.27	0.277	0.2735	89.1164	8 hours	control-super			
0.22	0.21	0.215	64.3647	8 hours	control-pellet	263.158		
0.222	0.222	0.222	67.2141	8 hours	control-pellet			
1.084	1.073	1.0785	569.592	8 hours	9 Gy-super	2237.86	2593.9	
1.068	1.032	1.05	549.337	8 hours	9 Gy-super			
0.28	0.259	0.2695	87.3588	8 hours	9 Gy-pellet	356.041		
0.308	0.246	0.277	90.6617	8 hours	9 Gy-pellet			

TNF ELISA

	1	2	3	4	5	6	7	8	9	
1	Standards									
2	1	2	3	4	5					
3	0	"	1693 (48°)	"	1705 (8°)					
4	15	"	1696	"	"					
5	31	"	1699	"	1715					
6	63	"	1700	"	"					
7	125	"	1703 (24°)	"	1718					
8	250	"	1711	"	"					
9	500	"	1712	"	1721					
10	1000	"	1714	"	"					
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										

Wave Length: 648.842 nm
 Path Length: 1.000 cm
 Read Mode: TRANSMITTANCE
 Build Mode: OFF
 0.000

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

$$y = 648.842 \times x^{1.84}$$

File

Edit Data

Linear Regression

Set fx= X

DATA:

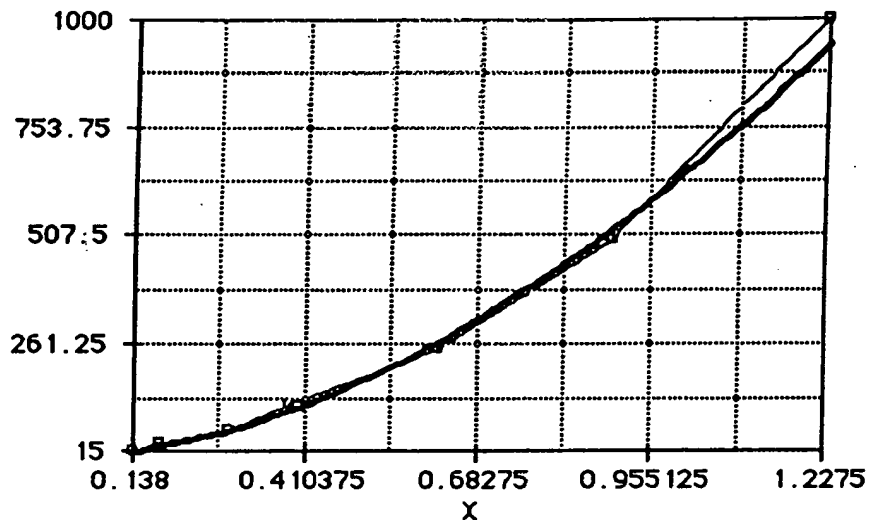
.....X.....Y
0.138	15.63
0.182	31.25
0.289	62.5
0.3845	125
0.623	250
0.8935	500
1.2275	1000

• EXPONENTIAL FIT •

$$Y = 648.841651 * (X)^{1.840847}$$

Coef. of Regression= 0.996618 For 7 Data Pairs

$$Y \quad f(x) = 648.841651 * (X)^{1.840847}$$



INITIALS GSS

SUBJECT • Untitled

DATE



CPU Time: 9 Secs.



TNF ELISA**In vivo**

reading 1	reading 2	average	[TNF]/ml	Time	Mouse #
0.339	0.207	0.273	59.52191	48 hours	1693
0.117	0.114	0.1155	12.22611	48 hours	1696
1.271	0.47	0.8705	502.7038	48 hours	1699
2.667	1.356	2.0115	2347.548	48 hours	1700
1.744	0.936	1.34	1111.758	24 hours	1703
0.758	0.319	0.5385	207.7396	24 hours	1711
0.328	0.218	0.273	59.52191	24 hours	1712
0.974	0.379	0.6765	316.1043	24 hours	1714
0.85	0.462	0.656	298.7038	8 hours	1701
0.148	0.106	0.127	14.55916	8 hours	1715
1.197	0.631	0.914	549.8935	8 hours	1718
0.572	0.47	0.521	195.4875	8 hours	1721

	1	2	3	4	5	6	7	8	9	
1	What we have:									
2	① 4 mice injected \bar{c} 10^6 V87 cells, now \bar{c} small tumors									
3	② 27 mice implanted \bar{c} 1mm^3 V87 implants									
4	③ 4 mice 5 tumors injected \bar{c} R3616									
5	④ 4 " " " " \bar{c} R899-6									
6										
7										
8	I. Compare 3616 & 899-6 in mice \bar{c} 1° V87 implants									
9	- Design: alternately inject tumors \bar{c} 4×10^7 PFU									
10	of R3616 or R899-6 when tumors reach 6 mm									
11	in greatest dimension.									
12	Measure tumors 2x/wk									
13	Sacrifice mice (i.e. 1 from each group on days 7, 14, 21, 28) for									
14	pathology?									
15										
16										
17	II. Use 2° passaged V87 implants (Need 48 mice - order 60)									
18	- Control + saline									
19	- RT (45Gy single dose) + saline									
20	- R3616 (4×10^7 PFU single dose)									
21	- R899-6 (4×10^7 PFU single dose)									
22	- RT (45Gy) + R3616									
23	- RT (45Gy) + R899-6									
24										
25	- Design: Take mice in groups of 4 when they									
26	reach 6mm in greatest dimension and randomly									
27	assign to a group. Use 8 mice per group.									
28	- Deliver RT 24° (?) + viral inoculation									
29	- Measure tumors 2x/wk									
30										
31										

$$\frac{2 \times 10^9}{4} = .025 \times 10^7$$

1	2	3	4	5	6	7	8	9
<p>III. In Vivo TNF assay i 2° passaged U87 tumors</p> <ul style="list-style-type: none">- R3616- R899-6- R3616 + RT- R899-6 + RT <p>- Design = inject tumors when reach 6 mm Sacrifice mice & assay tumors for TNF at specific intervals following inoculation: 12°, 24°, 36°, 48°, 60° (correct for [protein])</p> <p>IV. Use 4 mice i small tumors inject i <u>cells</u> ①</p> <ul style="list-style-type: none">- Sacrifice & assay tumor for TNF to serve as control for III.								

TNF V87

EFFICIENCY LINE 22-206

PURPOSE - To optimize intervals in order to test for RT inducibility

METHODS -

- ① V87 cells were grown to subconfluence in 60 mm plates
- ② Cells were infected with 10^6 PFU of R899-6 in 1.5 ml R99V media for 2°, then 2.5 ml of V87 media + IgG was added (total vol 4 ml)
- ③ Cells were incubated and irradiated with 8 Gy at the following intervals. The "supernatant" and "pellet" samples were aliquotted as in previous experiments.

Infect @ 6 ^{am} ⇒	RT time	Assay time
	None	6, 8, 10, 12, 14°
	6°	8, 10, 12, 14°
	8°	10, 12, 14°
	10°	12, 14°
	12°	14°

- ④ Prepare samples by freeze thaw x 3, then centrifuge
- ⑤ Measure human TNF α by ELISA

NAME: TNF CLARK #
 ADDRESS: Sibley/Hyland
 WAVELENGTH (nm) REFLECTANCE (%) SPLIT RATIO (%)
 SOL

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
B	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
C	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
D	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
E	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
F	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
G	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
H	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
I	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107
J	0.041	0.048	0.107	0.102	0.107	0.103	0.107	0.107	0.107	0.107	0.107	0.107

$$Y = 250.46 * X^{1.1968}$$

File

Edit Data

Linear Regression

Set fx=

X

DATA:

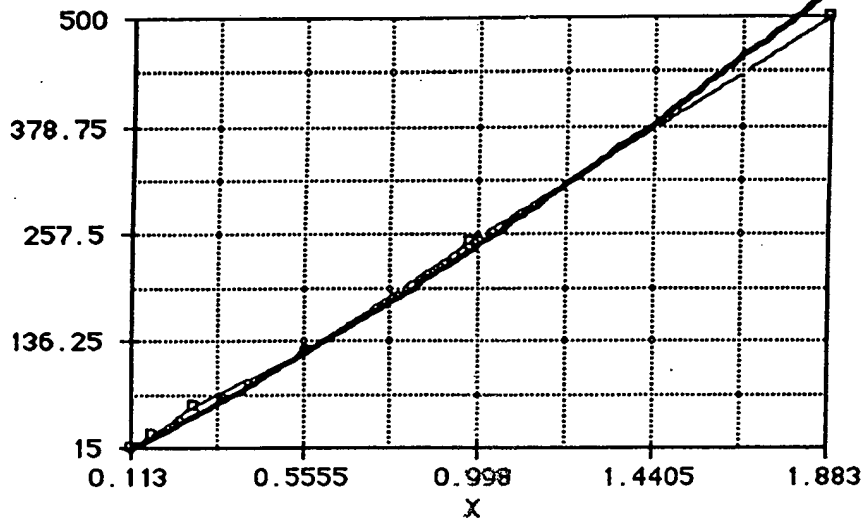
.....X.....Y.....
0.113	15.625
0.1645	31.25
0.2785	62.5
0.5645	125
0.981	250
1.883	500

• EXPONENTIAL FIT •

$$Y = 250.46143 * (X)^{1.196816}$$

Coef. of Regression= 0.993049 For 6 Data Pairs

$$Y \quad f(x) = 250.46143 * (X)^{1.196816}$$



INITIALS GSS

SUBJECT • Untitled

DATE ..

?

CPU Time: 11 Secs.

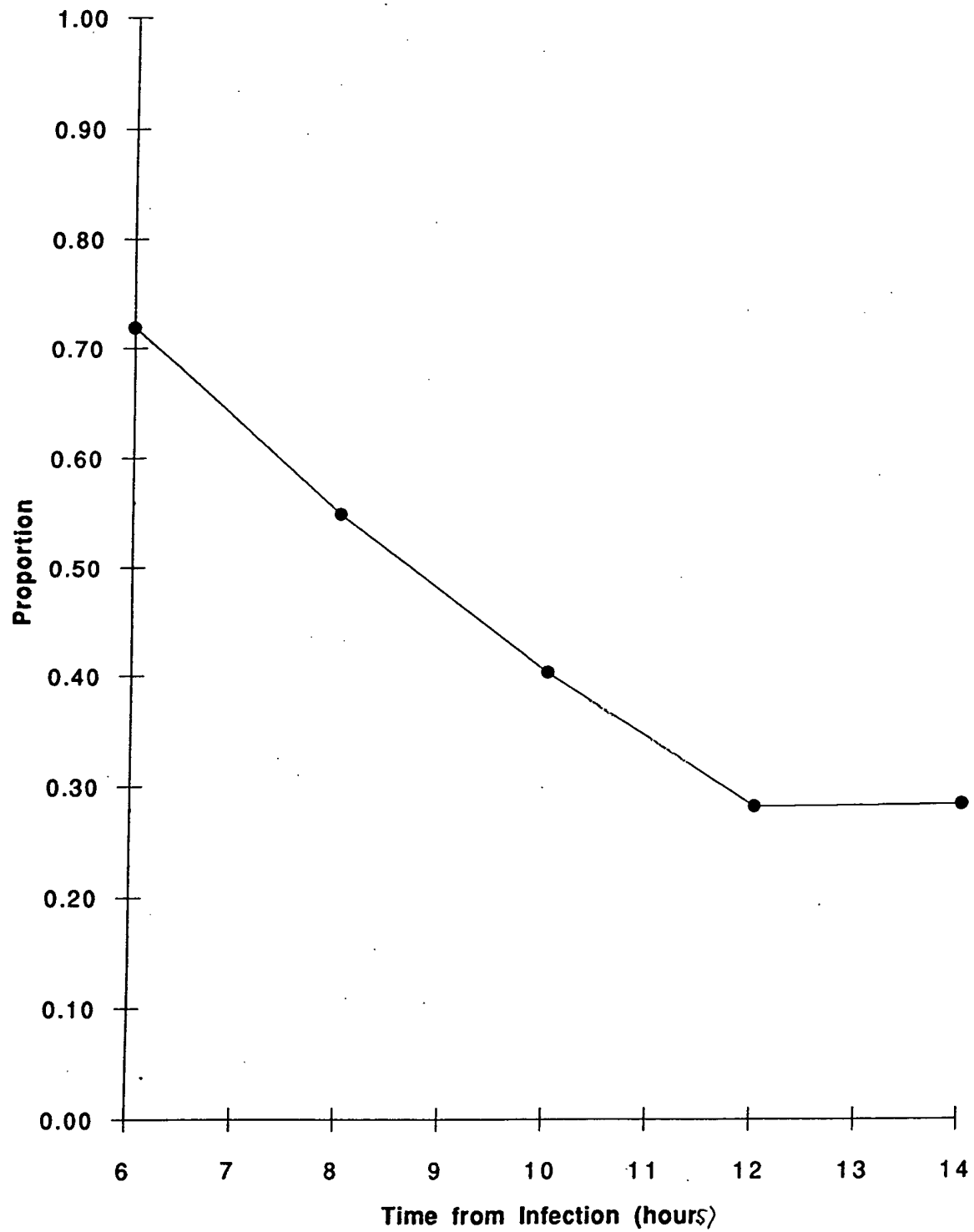


TNF ELISA

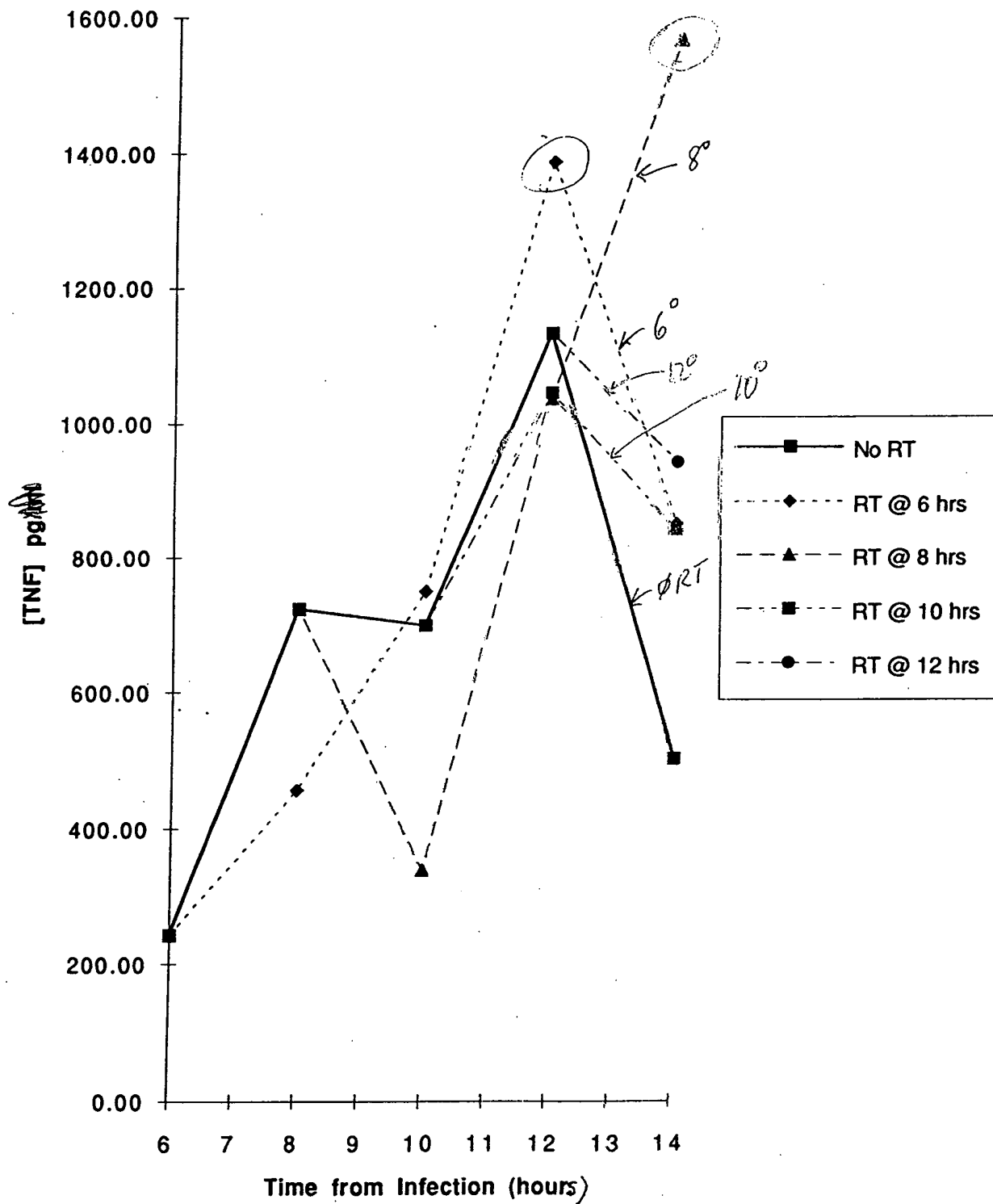
U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Total [TNF]
0.107	0.105	0.106	17.07	None	6 hours	supernatant	68.28	243.08
0.225	0.24	0.2325	43.70	None	6 hours	pellet	174.80	
0.389	0.395	0.392	81.66	None	8 hours	supernatant	326.62	723.19
0.46	0.462	0.461	99.14	None	8 hours	pellet	396.57	
0.294	0.299	0.2965	58.46	6 hours	8 hours	supernatant	233.84	456.40
0.281	0.288	0.2845	55.64	6 hours	8 hours	pellet	222.56	
0.484	0.478	0.481	104.31	None	10 hours	supernatant	417.24	699.51
0.349	0.345	0.347	70.57	None	10 hours	pellet	282.27	
0.267	0.262	0.2645	50.99	8 hours	10 hours	supernatant	203.97	339.52
0.188	0.188	0.188	33.89	8 hours	10 hours	pellet	135.55	
0.514	0.534	0.524	115.57	6 hours	10 hours	supernatant	462.27	749.90
0.339	0.366	0.3525	71.91	6 hours	10 hours	pellet	287.63	
0.817	0.865	0.841	203.58	None	12 hours	supernatant	814.32	1134.47
0.379	0.392	0.3855	80.04	None	12 hours	pellet	320.15	
0.755	0.767	0.761	180.63	10 hours	12 hours	supernatant	722.50	1046.13
0.388	0.39	0.389	80.91	10 hours	12 hours	pellet	323.63	
0.709	0.795	0.752	178.07	8 hours	12 hours	supernatant	712.29	1038.41
0.374	0.409	0.3915	81.53	8 hours	12 hours	pellet	326.12	
0.974	1.067	1.0205	256.62	6 hours	12 hours	supernatant	1026.47	1387.28
0.422	0.43	0.426	90.20	6 hours	12 hours	pellet	360.81	
0.431	0.419	0.425	89.95	None	14 hours	supernatant	359.79	503.15
0.196	0.198	0.197	35.84	None	14 hours	pellet	143.36	
0.72	0.737	0.7285	171.43	12 hours	14 hours	supernatant	685.73	943.84
0.316	0.328	0.322	64.53	12 hours	14 hours	pellet	258.11	
0.639	0.655	0.647	148.74	10 hours	14 hours	supernatant	594.96	843.50
0.308	0.316	0.312	62.14	10 hours	14 hours	pellet	248.54	
1.174	1.202	1.188	307.81	8 hours	14 hours	supernatant	1231.23	1566.84
0.399	0.403	0.401	83.90	8 hours	14 hours	pellet	335.62	
0.68	0.661	0.6705	155.23	6 hours	14 hours	supernatant	620.92	852.40
0.284	0.304	0.294	57.87	6 hours	14 hours	pellet	231.48	

Percentage of TNF in "Pellet"



TNF production by interval to RT



Greg's Mouse Log

Mouse #	Cage #	Tumor Date																
1698	AA104544	12	10	6	345	14	11	10	742.5	15	16.5	10						
1720	AA104544	4.5	3.5	3.5	27.563	6.5	6	4.5	87.75	9	9	5.5						
1723	AA104544	3.5	3.5	1.5	9.1875	3	3	2.5	11.25	4.5	5.5	2.5						
1724	AA104544	4	3.5	1.5	10.5	4	3	2	12	4.5	5	2						
1697	AA104549	5	4.5	3.5	39.375	5	4.5	4.5	50.625	6.5	8	5						
1702	AA104549	3	3	2	9	4.5	4	5	45	5	5.5	3						
1713	AA104549	5	5	3	37.5	6	5.5	5	82.5	4	9	5						
1704	AA104550	8.5	6.5	4.5	124.31	9.5	8	6	228	11.5	13.5	9						
1717	AA104550	7.3	6.5	4	84.25	6	6.5	5.5	107.25	9.5	8	7						
1719	AA104550	6	6	3.5	53	7	7	6	147	8	8.5	5						
1722	AA104550	7	6	4.5	94.5	7	6.5	6.5	147.88	9.5	8	4						
1707	AA105283	8	5	3.5	70	7	7.5	3	78.75	10.5	10.5	5						
1708	AA105283	4	4	2.5	20	3.5	3.5	2.5	15.313	6.5	6	4						
1709	AA105283	4	4	2	16	4	3	2	12	6.5	4.5	3						
1710	AA105283	5	4	3	30	6.5	6	4	78	9	8.5	5.5						

Mouse #	Cage #	Group	Infect Date	RT Date	Tumor size									
1720	AA104544	10 ⁹ 899-6												
1710	AA105283	10 ⁹ 899-6												
1707	AA105283	10 ⁹ 899-6												
1713	AA104549	10 ⁹ 899-6												
1698	AA104544													
1723	AA104544													
1724	AA104544													
1697	AA104549													
1702	AA104549													
1704	AA104550	Control												
1717	AA104550	Control												
1719	AA104550	Control												
1722	AA104550	Control												
1708	AA105283	10 ⁹ 899-6												
1709	AA105283	10 ⁹ 899-6												

283

283

549

549

wfs
 25.0
 21.7
 20.0
 23.0
 23.2
 24.5
 25.9
 25.2
 26.8
 24.8
 25.3
 24.5
 23.1
 26.8



THE UNIVERSITY OF CHICAGO
DEPARTMENT OF RADIATION & CELLULAR ONCOLOGY
DIVISION OF THE BIOLOGICAL SCIENCES AND
THE PRITZKER SCHOOL OF MEDICINE

Main Office: (312) 702-6819
Appointment Desk: (312) 702-6860
Facsimile: (312) 702-0610

University of Chicago Medical Center
5841 South Maryland Avenue, MC 0085
Chicago, Illinois 60637

Date:

To: James Linsley
From: *Gregory S. Sibley, MD*
Helena J. Mauceri

J-013.

Please transfer the cages listed below from CLSC 1053 to Carlson room J-019.
Please have the transfer completed by 5:00 p.m.
If this is not possible please call me at 2-0492.

Thank you.

Greg Sibley BP #3439
Cage numbers: EXT 2-0294

~~1859~~
~~AA108958 - AA108964~~ (7 cages)
AA10966 - AA108972
Mouse # 1859 - 1886

TNF U87 - in vitro

PURPOSE - To determine whether TNF production is
 Proot induced with 9 Gy RT following R899-6
 infection (10^6 PFU = \sim MOI = 0.5) in U87 cells.

METHODS - From previous experiments the optimal
 interval from infection to RT was 6 hrs. ~~And~~
 Optimal interval following RT was 6-8 hrs
 (total interval 12-14 hrs from infection.) ..

① See methods from 2/1/95. Briefly, 10^6 PFU of
 R899-6 was placed in 60 mm plates of
 subconfluent ($\sim 2 \times 10^6$ cells) U87 cells & incubate
 $\times 2^\circ$ in 15% V media @ 37° .

② 10% FCS Media + IgG added @ 2°

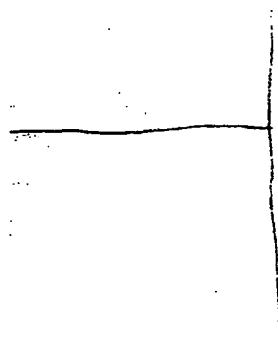
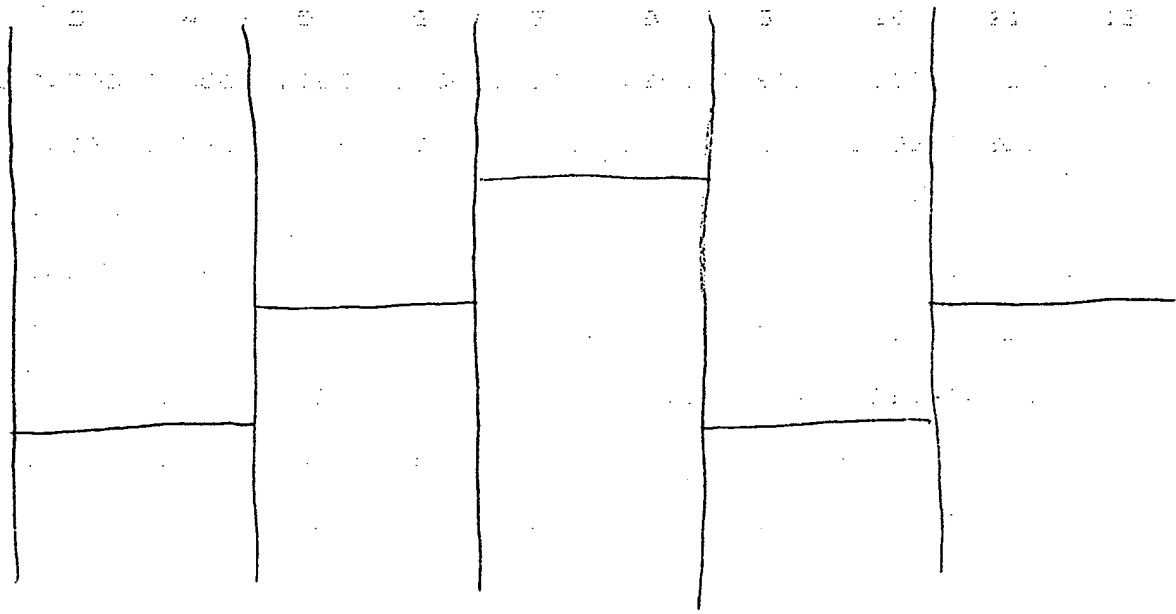
③ 9 Gy RT given @ 6° to RT group.

④ Plates sampled at 12 & 14 $^\circ$ for "supernatant" & "pellet"

TNF - U87
 Sidney / Hyland

$$Y = 216.779 X^{1.154631}$$

.0495





	1	2	3	4	5	6	7	8	9	
1	1-2	3-4	5-6	7-8	9-10	11-12	13-14			
2	0	12 S	12 P	12 RTS	14 S	14 P	"			
3	15	12 S	12 P	12 RTS	"	"	"			
4	31	12 S	12 P	12 RTP	"	"	14 RTP			
5	63	12 S	12 P	12 RTP	"	"	"			
6	125	12 S	12 RTS	12 RTP	"	14 RTS	"			
7	250	12 S	12 RTS	12 RTP	"	"	"			
8	500	12 P	12 RTS	12 RTP	14 P	"	"			
9	1000	12 P	12 RTS	12 RTP	"	"	"			
10										
11										
12										
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31										

$$\begin{array}{r} 314 \\ \times 8 \\ \hline 112 \\ \times 2 \\ \hline 224 \end{array}$$

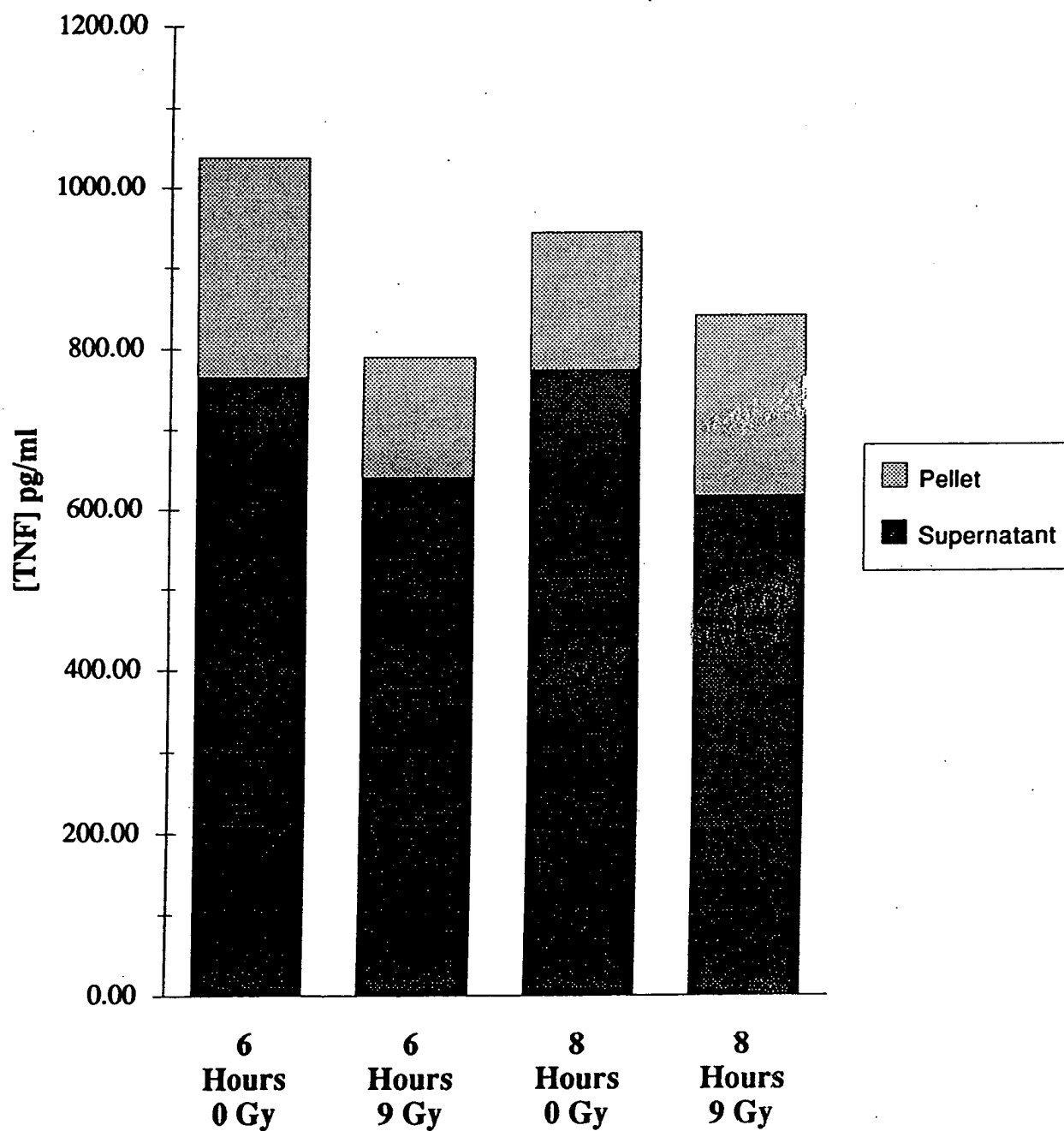
TNF ELISA

U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Ave [TNF]	Total TNF
0.908	0.896	0.902	192.44	none	12 hours	supernatant	769.76	764.69	1038.11
0.846	0.748	0.797	166.82	none	12 hours	supernatant	667.26		
0.892	0.847	0.8695	184.46	none	12 hours	supernatant	737.83		
0.857	0.842	0.8495	179.57	none	12 hours	supernatant	718.27		
0.936	0.945	0.9405	201.96	none	12 hours	supernatant	807.82		
1.014	1.026	1.02	221.79	none	12 hours	supernatant	887.17		
0.368	0.372	0.37	68.78	none	12 hours	pellet	275.11	273.43	
0.383	0.386	0.3845	71.90	none	12 hours	pellet	287.60		
0.363	0.36	0.3615	66.96	none	12 hours	pellet	267.83		
0.392	0.377	0.3845	71.90	none	12 hours	pellet	287.60		
0.366	0.33	0.348	64.08	none	12 hours	pellet	256.31		
0.368	0.351	0.3595	66.53	none	12 hours	pellet	266.12		
0.824	0.884	0.854	180.67	6 hours	12 hours	supernatant	722.66	640.45	789.94
0.791	0.835	0.813	170.69	6 hours	12 hours	supernatant	682.76		
0.833	0.821	0.827	174.09	6 hours	12 hours	supernatant	696.35		
0.734	0.721	0.7275	150.14	6 hours	12 hours	supernatant	600.54		
0.693	0.8	0.7465	154.67	6 hours	12 hours	supernatant	618.69		
0.602	0.686	0.644	130.42	6 hours	12 hours	supernatant	521.69		
0.234	0.251	0.2425	42.23	6 hours	12 hours	pellet	168.91	149.49	
0.188	0.219	0.2035	34.49	6 hours	12 hours	pellet	137.95		
0.21	0.241	0.2255	38.83	6 hours	12 hours	pellet	155.31		
0.234	0.222	0.228	39.33	6 hours	12 hours	pellet	157.30		
0.209	0.21	0.2095	35.66	6 hours	12 hours	pellet	142.66		
0.203	0.196	0.1995	33.71	6 hours	12 hours	pellet	134.82		
0.876	0.93	0.903	192.69	none	14 hours	supernatant	770.75	774.25	943.63
0.813	0.792	0.8025	168.15	none	14 hours	supernatant	672.58		
0.935	0.947	0.941	202.08	none	14 hours	supernatant	808.32		
0.924	0.9	0.912	194.91	none	14 hours	supernatant	779.63		
0.969	0.953	0.961	207.05	none	14 hours	supernatant	828.19		
0.924	0.913	0.9185	196.51	none	14 hours	supernatant	786.04		
0.248	0.249	0.2485	43.44	none	14 hours	pellet	173.74	169.38	
0.27	0.271	0.2705	47.90	none	14 hours	pellet	191.62		
0.251	0.254	0.2525	44.24	none	14 hours	pellet	176.97		
0.233	0.24	0.2365	41.02	none	14 hours	pellet	164.09		
0.244	0.224	0.234	40.52	none	14 hours	pellet	162.09		
0.211	0.221	0.216	36.95	none	14 hours	pellet	147.78		
0.65	0.653	0.6515	132.18	6 hours	14 hours	supernatant	528.71	616.03	840.68
0.722	0.696	0.709	145.74	6 hours	14 hours	supernatant	582.95		
0.62	0.555	0.5875	117.30	6 hours	14 hours	supernatant	469.21		
0.775	0.784	0.7795	162.59	6 hours	14 hours	supernatant	650.38		

0.886	0.814	0.85	179.69	6 hours	14 hours	supernatant	718.76	
0.895	0.861	0.878	186.54	6 hours	14 hours	supernatant	746.16	
0.314	0.294	0.304	54.82	6 hours	14 hours	pellet	219.27	224.66
0.307	0.303	0.305	55.03	6 hours	14 hours	pellet	220.11	
0.296	0.31	0.303	54.61	6 hours	14 hours	pellet	218.44	
0.295	0.307	0.301	54.19	6 hours	14 hours	pellet	216.78	
0.315	0.312	0.3135	56.80	6 hours	14 hours	pellet	227.20	
0.343	0.329	0.336	61.53	6 hours	14 hours	pellet	246.14	

TNF Production By RT in U87 Cells Following R899-6 Infection



Greg's Mouse Log

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	weight	Tumvol	weight	Tumvol	weight
1704	AA104550	Control		None	None	14	14.5	13	0	22.1
1717	AA104550	Control		None	None	12.5	11.5	9.5	0	23.9
1719	AA104550	Control		None	None	10.5	12	7	0	21.7
1722	AA104550	Control		None	None	13	14	10	0	25
1720	AA105283	10^7 899-6			None	11	12	8	0	25.1
1710	AA105283	10^7 899-6			None	13	14.5	7.5	0	21.6
1707	AA105283	10^7 899-6			None	15.5	15	8.5	0	18.1
1713	AA105283	10^7 899-6			None	7.5	7.5	3.5	0	23.2
1697	AA104549	10^7 3616			None	11	11	7	0	24.2
1708	AA104549	10^7 3616			None	9.5	9	6	0	24.0
1709	AA104549	10^7 3616			None	9.5	10	6.5	0	27.7
1723	AA104549	10^7 3616			None	8.5	8	5.5	0	25.3
1859	AA108972	RT		None		10	9	6	0	24.4
1860	AA108972	RT		None		13	11.5	7	0	20.2
1861	AA108972	RT		None		9.5	10	6	0	19.1
1862	AA108972	RT		None		12	8.5	7	0	22.9
1702	AA104544					6	6.5	3.5	1	26.5
1724	AA104544					7	5.5	3.5	1	24
1863	AA108971	Large		36/1/1/1		10	7	6.5	21.3	
1864	AA108971	Large				12	7	5.5	21.9	
1865	AA108971	Large				8.5	7	5	22.6	
1866	AA108971	Large				9.5	6.5	5.5	23.0	
1867	AA108970	Large		RT Alone		8	6	5		24.7
1868	AA108970	Large		"		6.5	7	6.5		21.2
1869	AA108970	Large		"		6.5	7	4.5		22.7
1870	AA108970	Large		"		9	7	6		22.7
1871	AA108969	Large 899-6		"		8	6	5		22.2
1872	AA108969	Large 1141		"		8.5	7	6.5		20.2
1873	AA108969	Large		"		6.5	6	6.5		21.4
1874	AA108969	Large "		"		10.5	9	6		24.5

Titers

New Samples : Vins used for all in vivo experi.

Vins	Dilution	Count	Titer
3616	10^7	Tm	Tm
	10^8	23	2.6×10^9
899-6	10^7	Tm	Tm
	10^8	29	3.8×10^9

Old samples (used for in vitro experi.)

Vins	Dilution	Count	Titer
3616	10^7	Tm	Tm
	10^8	45	4.1×10^9
899-6	10^7	Tm	Tm
	10^8	35	3.1×10^9

Greg's Mouse Log

Mouse #	Cage #	Group	weight	Tumvol	weight	Tumvol	weight
1704	AA104550	Control	21	11	0	21.7	0
1717	AA104550	Control	13	12.5	10.5	0	0
1719	AA104550	Control	13	11.5	6	0	0
1722	AA104550	Control	14	13.5	10	0	0
1867	AA108970	Control	10	8	6		
1868	AA108970	Control	10	9.5	7.5		
1869	AA108970	Control	10	9	7		
1870	AA108970	Control	10.5	8.5	8		
		Control	Mean:	0		Mean:	0
			Std Error:	0		Std Error:	0
			Day:	17		Day:	0
1720	AA105283	10^7 899-6	12	11	7.5	0	0
1710	AA105283	10^7 899-6	16	13.5	7	0	0
1707	AA105283	10^7 899-6	16	15	11	0	0
1713	AA105283	10^7 899-6	6.5	5.5	3	0	0
		10^7 899-6	Mean:	0		Mean:	0
			Std Error:	0		Std Error:	0
			Day:	17		Day:	0
1697	AA104549	10^7 3616	10	9	7	0	0
1708	AA104549	10^7 3616	8	8.5	5	0	0
1709	AA104549	10^7 3616	9	10	7	0	0
1723	AA104549	10^7 3616	7.5	9	6	0	0
		10^7 3616	Mean:	0		Mean:	0
			Std Error:	0		Std Error:	0
			Day:	17		Day:	0
1859	AA108972	RT Alone	5.5	4.5	5.5	0	0
1860	AA108972	RT Alone	12	10.5	8	0	0
1861	AA108972	RT Alone	10.5	9	7	0	0
1862	AA108972	RT Alone	12	9	7	0	0
		RT Alone	Mean:	0		Mean:	0
			Std Error:	0		Std Error:	0
			Day:	17		Day:	0

[illegible]

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 18781

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASIS

REQUEST BY: Greg Sibley, MD DATE: _____

REQUESTOR'S PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: _____

FAS ACCOUNT: 2-7371-5100

P.I.: Hallan

VENDOR: FCR1

PROTOCOL: 58671

REQUESTED DELIVERY DATE: _____

PHONE: 2-0294

SPECIES: Mouse

QUANTITY: 60

STRAIN: Hymanic Nude

SEX: M ☒ F EITHER

WEIGHT/AGE: 25-6 wks ALTERNATE WEIGHT/AGE: _____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER _____ CLSC ☒ FMI _____ OTHER _____



The University of Chicago
Departmental Purchase Order

Greg

Z 873342

THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

Vendor Name USA Orders - Cust # 112486
Life Technologies, Inc
P.O. Box 68
Grand Island State NY Zip Code 14072
Payment Terms _____ Delivery charge? ☐ Yes ☒ No
Telephone No. 1-800-828-6686 FAX No. 1-800-331-2286

Ship to THE UNIVERSITY OF CHICAGO Dept. Code: _____
Radiation Oncology MC-0085
Greg Sibley, MD Department _____ Room G-03
5830 S. Ellis Ave
Chicago State IL Zip Code 60637

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services as stated in University policies & procedures; chaining, are used to exceed the restriction of \$500.00 for one purchase; travel, any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filed out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

372-369

Order placed by phone? ☐ No ☐ Yes
Melissa Ref # 372369 Date _____
Greg Sibley, MD Phone # _____
Order placed with (name) _____
Order placed by (name) _____

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
24		11095-023 Minimal Essential Media 500 ml	8.64	207.36
12		15140-015 Penicillin - Streptomycin 100 ml	10.00	250.00
18		22400-055 RPMI 1640 Media 500 ml		120.00
12		22400-055 RPMI 1640 Media 500 ml	12.91	154.92
				482.28

ORDER TOTAL

NOT VALID IF TOTAL EXCEEDS \$500.00

DEPARTMENT COPY

BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

Greg's Mouse Log

Mouse #	Cage #	Group	Mean	Std Error	Day	Tumvol	weight	Mean	Std Error	Day	Tumvol	weight
1704	AA104550	Control										
1717	AA104550	Control	18	15.5	14	0					0	
1719	AA104550	Control	12	15	8	0					0	
1722	AA104550	Control	19.5	18	13	0					0	
1867	AA108970	Control	13	16	9.5							
1868	AA108970	Control	18	15	13							
1869	AA108970	Control	15.5	16	9							
1870	AA108970	Control	15.5	13	11							
		Control	Mean:			0		Mean:				
			Std Error:			0		Std Error:				
			Day:			17		Day:				
1720	AA105283	10^7 899-6	11.5	11.5	8	0						
1710	AA105283	10^7 899-6	14.5	16	8	0						
1707	AA105283	10^7 899-6	13	16.5	13	0						
1713	AA105283	10^7 899-6				0						
		10^7 899-6	Mean:			0		Mean:				
			Std Error:			0		Std Error:				
			Day:			17		Day:				
1697	AA104549	10^7 3616	11	8.5	5.5	0						
1708	AA104549	10^7 3616	5.5	7	3.5	0						
1709	AA104549	10^7 3616	6	7.5	4	0						
1723	AA104549	10^7 3616	9	9	5	0						
		10^7 3616	Mean:			0		Mean:				
			Std Error:			0		Std Error:				
			Day:			17		Day:				
1859	AA108972	RT Alone	10	11.5	6.5	0						
1860	AA108972	RT Alone	9	8	6.5	0						
1861	AA108972	RT Alone	11.5	12.5	8	0						
1862	AA108972	RT Alone	11	9	4	0						
		RT Alone	Mean:			0		Mean:				
			Std Error:			0		Std Error:				
			Day:			17		Day:				

[illegible]

5 5.9 5.8

				Std Error:	#DIV/0!		Std Error:	#DIV/0!
				Day:	0		Day:	0
1891	AA108964	pc A6ne	6.5	7	5.5			
1892	AA108964	"	8	9	5			
1893	AA108964	"	9.5	7.5	7			
1894	AA108964	"	8	8.5	6			
1895	AA108963							
1896	AA108963							
1897	AA108963							
1898	AA108963							
1899	AA108962							
1900	AA108962							
1901	AA108962							
1902	AA108962							
1903	AA108961							
1904	AA108961							
1905	AA108961							
1906	AA108961							
1907	AA108960							
1908	AA108960							
1909	AA108960							
1910	AA108960							
1911	AA108959							
1912	AA108959							
1913	AA108959							
1914	AA108959							
1915	AA108958							
1916	AA108958							
1917	AA108958							
1918	AA108958							
1702	AA104544							
1724	AA104544							

Jim Linsley
P-110
Animal Resources

Dear Jim,

Please transfer the cages listed below from CLSC 1053 to Carlson room J-O13.
Please have the transfer completed by 5 pm on

If this is not possible, please contact me.

Thank you,

Greg Sibley, M.D.
Dept. of Radiation and Cellular Oncology
Beeper #3439
Extension 2-0294

Cage Numbers:
AA111326 through AA111333

Total Number of Cages: 8

Greg's Mouse Log						
Mouse #	Cage #	Group				
720	AA105283	10^7 899-6	+17.5	12	-1.5	
1710	AA105283	10^7 899-6	+6.5	6.5	-1.5	
1713	AA105283	10^7 899-6	+0	0	0	
		10^7 899-6				
1697	AA104549	10^7 3616	+15.5	13.5	2	
1708	AA104549	10^7 3616	+2	2.5	-1.5	
1709	AA104549	10^7 3616	+5	5	1	
1723	AA104549	10^7 3616	+0	0	0	
1875	AA108968	10^7 3616	+19	18.5	10	
1876	AA108968	10^7 3616	+18	17	11.5	
1877	AA108968	10^7 3616	+18.5	14	13	
1878	AA108968	10^7 3616	+16	12.5	11	
		10^7 3616				
1859	AA108972	RT Alone	7	1.5	4	
1860	AA108972	RT Alone 13x11x6	8.5	7.5	5	
1861	AA108972	RT Alone	10	10.5	7	
1862	AA108972	RT Alone	8.5	7.5	3	
1891	AA108964	RT Alone	+6.5	6.5	5	
1892	AA108964	RT Alone	+12.5	7.5	6	
1893	AA108964	RT Alone	+10	7.5	5.5	
1894	AA108964	RT Alone	+8	8	5	
		RT Alone				
1863	AA108971	10^7 3616+RT	+10	8	4	
1864	AA108971	10^7 3616+RT	+10.5	7	4	
1865	AA108971	10^7 3616+RT	+5.5	6.5	4.5	
1866	AA108971	10^7 3616+RT	+7	5	4.5	
1883	AA108966	10^7 3616+RT	+3.5	3	1.5	
1884	AA108966	10^7 3616+RT	+5	8	6	
1885	AA108966	10^7 3616+RT	+7	8.5	4	
1886	AA108966	10^7 3616+RT	+3.5	4	2	
		10^7 3616+RT				

1871	AA108969	10^7 899-6+RT	7.5	7	8.5
1872	AA108969	10^7 899-6+RT	7	5.5	4.5
1873	AA108969	10^7 899-6+RT	8	7.5	4.5
1874	AA108969	10^7 899-6+RT	8	7.5	4
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	3.5	3	1
1881	AA108967	10^7 899-6+RT	5.5	6	3
1882	AA108967	10^7 899-6+RT	4	3	1
		10^7 899-6+RT			
2032	AA111326	10^7 899-6			
2033	AA111326	10^7 899-6			
2034	AA111326	10^7 899-6			
2035	AA111326	10^7 899-6			
2036	AA111327	10^7 899-6			
2037	AA111327	10^7 899-6			
2038	AA111327	10^7 899-6			
2039	AA111327	10^7 899-6			
2040	AA111328	10^7 899-6			
2041	AA111328	10^7 899-6			
2042	AA111328	10^7 899-6			
2043	AA111328	10^7 899-6			
		10^7 899-6			
2044	AA111329	10^7 3616			
2045	AA111329	10^7 3616			
2046	AA111329	10^7 3616			
2047	AA111329	10^7 3616			
2048	AA111330	10^7 3616			
2049	AA111330	10^7 3616			
2050	AA111330	10^7 3616			
2051	AA111330	10^7 3616			
		10^7 3616			
2052	AA111331	10^7 899-6+RT			
2053	AA111331	10^7 899-6+RT			
2054	AA111331	10^7 899-6+RT			
2055	AA111331	10^7 899-6+RT			
2056	AA111332	10^7 899-6+P			
2057	AA111332	10^7 899-6			
2058	AA111332	10^7 899-6			
2059	AA111332	10^7 899-6			
		10^7 899-6+h.			

[illegible]

[illegible]

1871	AA108969	10^7 899-6+RT										
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT										
1874	AA108969	10^7 899-6+RT										
1879	AA108967	10^7 899-6+RT										
1880	AA108967	10^7 899-6+RT										
1881	AA108967	10^7 899-6+RT										
1882	AA108967	10^7 899-6+RT										
		10^7 899-6+RT										
2032	AA111326	10^7 899-6 RT	9	7	6		22.2	3616	+RT			
2033	AA111326	10^7 899-6 RT	11	7.5	6.5		22.6	"				
2034	AA111326	10^7 899-6 RT	11	10	7		19.6	"				
2035	AA111326	10^7 899-6 RT	12.5	7	6		19.0	"				
2036	AA111327	10^7 899-6 RT	10	7	5		19.7	3616	+RT			
2037	AA111327	10^7 899-6 RT	8	7	4.5		21.7	"				
2038	AA111327	10^7 899-6 RT	11.5	7.5	7.5		13.0	"				
2039	AA111327	10^7 899-6 RT	9.5	7	5		15.5	"				
2040	AA111328	10^7 899-6	11	9.5	7		21.3	3616	6			
2041	AA111328	10^7 899-6	6.5	6	5		21.3	899-6+RT	11			
2042	AA111328	10^7 899-6	10	10	7.5		23.2	"				
2043	AA111328	10^7 899-6	8	6.5	5.5		23.7	"				
		10^7 899-6										
2044	AA111329	10^7 3616+RT	8.5	7	4.5		19.3	899-6	+RT			
2045	AA111329	10^7 3616+RT	7	6	4		22.4	"				
2046	AA111329	10^7 3616 "	6.5	6	5		20.7	"				
2047	AA111329	10^7 3616 "	9	8.5	5		21.5	"				
2048	AA111330	10^7 3616+RT	8	5	4.5		19.2	899-6	+RT			
2049	AA111330	10^7 3616 "	11	7	5.5		22.6	"				
2050	AA111330	10^7 3616 "	8.5	6.5	5.5		21.1	"				
2051	AA111330	10^7 3616 "	8.5	8.5	5.5		19.2	"				
		10^7 3616										
2052	AA111331	10^7 899-6+RT	7	6	5		21.4	899-6				
2053	AA111331	10^7 899-6+RT	7	6	4.5		21.7	899-6				
2054	AA111331	10^7 899-6+RT	7	6	4.5		22.1	"				
2055	AA111331	10^7 899-6+RT	"	6.5	5		23.3	"				
2056	AA111332	10^7 899-6+RT	8	5	4		21.7	899-6				
2057	AA111332	10^7 899-6+RT	7	6	4.5		21.8	"				
2058	AA111332	10^7 899-6+RT	7	7	5.5		22.4	"				
2059	AA111332	10^7 899-6+RT						"				
		10^7 899-6+RT										

extra injected

Bubba Tumors

1331

1 tumor

1332

1 tumor

6/15/68 8:55 AM

[illegible]

Greg's Mouse Log						
Mouse #	Cage #	Group			Tumvol	weight
720	AA105283	10^7 899-6	-20.5	10	13	26.0
1710	AA105283	10^7 899-6	-0	0	0	23.0
1713	AA105283	10^7 899-6	-0	0	0	21.6
		10^7 899-6				
1697	AA104549	10^7 3616	+17.5	4.5	11	23.8
1708	AA104549	10^7 3616	+0	0	0	24.0
1709	AA104549	10^7 3616	+3.5	4	1	28.2
1723	AA104549	10^7 3616	+0	0	0	26.3
1875	AA108968	10^7 3616	+19	20	10	22.3
1876	AA108968	10^7 3616	+17	17	13.5	23.5
1877	AA108968	10^7 3616	+22.5	17.5	16.5	27.3
1878	AA108968	10^7 3616	+18.5	15.5	15.5	26.0
		10^7 3616				
1859	AA108972	RT Alone	10	10	5	25.1
1860	AA108972	RT Alone	13	1	8.5	8.7
1861	AA108972	RT Alone	11	1.5	7	20.9
1862	AA108972	RT Alone	9	7.5	3.5	24.7
1891	AA108964	RT Alone	6.5	7.5	3.5	19.8
1892	AA108964	RT Alone	7.5	9.5	5	21.5
1893	AA108964	RT Alone	10	9	5.5	20.8
1894	AA108964	RT Alone	7.5	8	4	21.5
		RT Alone				
863	AA108971	10^7 3616+RT	+9.5	8	3.5	22.9
864	AA108971	10^7 3616+RT	+6	5	4	25.3
865	AA108971	10^7 3616+RT	+10	6.5	4	24.9
866	AA108971	10^7 3616+RT	+5	4	3.5	25.1
883	AA108966	10^7 3616+RT	+4	3	1	21.7
884	AA108966	10^7 3616+RT	+3.5	7	4.5	23.5
885	AA108966	10^7 3616+RT	Dead	3/14/45		
886	AA108966	10^7 3616+RT	+3	4	4	22.5
		10^7 3616+RT				

[illegible]

[illegible]

Greg's Mouse Log					
Mouse #	Cage #	Group			
			Tumvol	weight	
720	AA105283	10^7 899-6	21	15	11
1710	AA105283	10^7 899-6	0	0	0
1713	AA105283	10^7 899-6	0	0	0
		10^7 899-6			
1697	AA104549	10^7 3616	18	16	10
1708	AA104549	10^7 3616	0	0	0
1709	AA104549	10^7 3616	3.5	3.5	1
1723	AA104549	10^7 3616	2	2	1
1875	AA108968	10^7 3616	17.5	19	8
1876	AA108968	10^7 3616	20	20.5	13.5
1877	AA108968	10^7 3616	24	18	17
1878	AA108968	10^7 3616	20.5	17.5	13.5
		10^7 3616			
1859	AA108972	RT Alone	9.5	10	3.5
1860	AA108972	RT Alone	13	12.5	5.5
1861	AA108972	RT Alone	11.5	12	5.5
862	AA108972	RT Alone	7	7	4
891	AA108964	RT Alone	6	6.5	3.5
892	AA108964	RT Alone	12.5	9	6
893	AA108964	RT Alone	9.5	8	4.5
894	AA108964	RT Alone	7	7	3.5
		RT Alone			
863	AA108971	10^7 3616+RT	9.5	8	3
864	AA108971	10^7 3616+RT	9	6.5	2
865	AA108971	10^7 3616+RT	5	3.5	3
866	AA108971	10^7 3616+RT	3	3	1
883	AA108966	10^7 3616+RT	3.5	2	1
884	AA108966	10^7 3616+RT	8.5	7	5.5
885	AA108966	10^7 3616+RT	2.5	3	1
886	AA108966	10^7 3616+RT	2.5	3	1
		10^7 3616+RT			

2

1871	AA108969	10^7 899-6+RT	6	6	3.5
1872	AA108969	10^7 899-6+RT	5.5	5	3
1873	AA108969	10^7 899-6+RT	7	7	3.5
1874	AA108969	10^7 899-6+RT	7.5	7.5	3
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	0	0	0
1881	AA108967	10^7 899-6+RT	5.8	6	2.5
1882	AA108967	10^7 899-6+RT	3	2.5	1
		10^7 899-6+RT			
2032	AA111326	10^7 899-6	11	8	6
2033	AA111326	10^7 899-6	13	7	6
2034	AA111326	10^7 899-6	11.5	11	7
2035	AA111326	10^7 899-6	12.5	8.5	8
2036	AA111327	10^7 899-6	11.5	7.5	6
2037	AA111327	10^7 899-6	9.5	8	5.5
2038	AA111327	10^7 899-6	13	11	8.5
2039	AA111327	10^7 899-6	10.5	8.5	6
2040	AA111328	10^7 899-6	13	12	8
2041	AA111328	10^7 899-6	8	7.5	5.5
2042	AA111328	10^7 899-6	12	14	11
2043	AA111328	10^7 899-6	11	8.5	6
		10^7 899-6			
2044	AA111329	10^7 3616	11	9	7
2045	AA111329	10^7 3616	8	6.5	4.5
2046	AA111329	10^7 3616	9	7.5	6
2047	AA111329	10^7 3616	10.5	10.5	5
2048	AA111330	10^7 3616	9	6	4.5
2049	AA111330	10^7 3616	15	8	6.5
2050	AA111330	10^7 3616	9	7.5	5.5
2051	AA111330	10^7 3616	11	10	5.5
		10^7 3616			
2052	AA111331	10^7 899-6+RT	10	7.5	6.5
2053	AA111331	10^7 899-6+RT	6.5	8	6
2054	AA111331	10^7 899-6+RT	9.5	7.5	4.5
2055	AA111331	10^7 899-6+RT	14	7.5	6.5
2056	AA111332	10^7 899-6+RT	10	7	4.5
2057	AA111332	10^7 899-6+RT	7.5	7	5
2058	AA111332	10^7 899-6+RT	10	12	5
2059	AA111332	10^7 899-6+RT			
		10^7 899-6+RT			

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0			weight	Day 3	weight
1720	AA105283	10^7 899-6	23	10	14	25.2		
1710	AA105283	10^7 899-6	0	0	0	23.3		
1713	AA105283	10^7 899-6	0	0	0	22.1		
2052	AA111331	10^7 899-6	11	9.5	6	18.4		
2053	AA111331	10^7 899-6	8	10.5	7	21.1		
2054	AA111331	10^7 899-6	7	6	4.5	19.5		
2055	AA111331	10^7 899-6	10	12	7.5	17.4		
2056	AA111332	10^7 899-6	9	7	5	23.4		
2057	AA111332	10^7 899-6	7.5	6	4.5	21.8		
2058	AA111332	10^7 899-6	11	7.5	5.5	23.5		
2060	AA111333	10^7 899-6	11	8.5	7	22.3		
2061	AA111333	10^7 899-6	10	9	7	23.3		
2062	AA111333	10^7 899-6	9	7.5	6.5	18.0		
2063	AA111333	10^7 899-6	9.5	7.5	5.5	20.5		
		10^7 899-6						
1697	AA104549	10^7 3616	18	15	10.5	23.4		
1708	AA104549	10^7 3616	5	3	1	21.5		
1709	AA104549	10^7 3616	3	3	1	27.6		
1723	AA104549	10^7 3616	0	0	0	26.1		
1875	AA108968	10^7 3616	20.5	16.5	1	18.3	→ 50 L	
1876	AA108968	10^7 3616	Dead					
1877	AA108968	10^7 3616	26	19	13	26.3		
1878	AA108968	10^7 3616	22	13.5	17	23.8		
2040	AA111328	10^7 3616	16	14	8.5	22.2	→ AA 111 335	
2041	AA111328	10^7 3616	6	5.5	4	22.2		
2042	AA111328	10^7 3616	17	17	12	23.6	→ "	
2043	AA111328	10^7 3616	10.5	9	5	24.0		
2064	AA111334	10^7 3616	9.5	7	7	21.4		
2065	AA111334	10^7 3616	8.3	8.3	10	21.1		
2066	AA111334	10^7 3616	11	7.5	6	16.5		
2067	AA111334	10^7 3616	11	7.5	8	22.2		
		10^7 3616						
2070			10.5	7	6	23.3		
1859	AA108972	RT Alone	14.5	14	11	18.5		
1860	AA108972	RT Alone	8	10	5.5	24.0		
1861	AA108972	RT Alone	11.5	12	7.5	21.0		
1862	AA108972	RT Alone	8	6.5	3.5	23.5		
1891	AA108964	RT Alone	Dead					
1892	AA108964	RT Alone	8	8	5	19.2		
1893	AA108964	RT Alone	Dead					
1894	AA108964	RT Alone	Dead					
		RT Alone						

[illegible]

IN VIVO TNF INDUCTION

PURPOSE - To see if TNF α induction production is induced in vivo with 20 Gy delivered 24^o after injection of U87 hind limb tumors with R899-6 (10^7 PFU)

METHODS

1. Implant subcut tumors in hind limb of nude mice
2. Grow to $\sim 100 \text{ mm}^3$ in size
3. Inject with 2×10^7 PFU R899-6 (10 μ l of stock)
4. 24 hours after injection randomize to 4-20 Gy, and sacrifice at the following intervals:

Time	⊖ RT	⊕ RT	
2 ^o + 24 ^o	4 mice	4 mice	Need 64 mice
→ 6 ^o + 24 ^o	"	"	
→ 12 ^o + 24 ^o	"	"	Infect W 9am
→ 24 ^o + 24 ^o	"	"	RT Th 9am
→ 48 ^o + 24 ^o	"	"	Sac @ 3pm
→ 1wk + 24 ^o	"	"	9am
→ 2wk + 24 ^o	"	"	F 9am
3wk + 24 ^o	"	"	

INDUCTION- in vivo							Note: Old standard curve data used				
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor	Tot Vol	TNF/ml	TNF/tumor	Mean	TNF/mg-tum	Mean
1702	Control	None		63	0.06	0.66	18	12	817	199	703
1698	Control	None		2520	2.5	3.5	477	1668		667	
1724	Control	None		67	0.07	0.67	23	15		219	
2080	Control 1	None		938	0.9	1.5	259	388		432	
2081	Control 2	None		1124	1	2	1000	2000		2000	
2042	3616			528	0.6	1.2	252	303	261	505	408
2034	3616+20+25			644	0.7	1.3	168	218		312	
2077	899-6			2090	2.9	3.9	56	219	664	75	161
2078	899-6			6186	4.5	5.5	202	1109		246	
2145	2 hrs + RT				0.51	1.11	214	238	127	466	573
2146	2 hrs + RT				0.39	0.99	81	80		206	
2147	2 hrs + RT				0.06	0.66	95	63		1047	
2161	6 hrs + RT				0.7	1.3	158	205	156	293	271
2162	6 hrs + RT				0.66	1.26	144	182		276	
2163	6 hrs + RT				0.68	1.28	124	158		233	
2164	6 hrs + RT				0.28	0.88	90	79		283	
2177	6 hrs no RT				1.03	2.03	246	499	261	484	663
2178	6 hrs no RT				0.5	1.1	138	151		303	
2179	6 hrs no RT				0.23	0.83	191	159		690	
2180	6 hrs no RT				0.2	0.8	293	235		1174	
2185	12 hrs + RT				0.5	1.1	196	216	158	431	307
2186	12 hrs + RT				0.54	1.14	83	95		176	
2187	12 hrs + RT				0.47	1.07	81	87		185	
2188	12 hrs + RT				0.54	1.14	206	235		436	
2141	12 hrs no RT				0.19	0.79	122	96	111	506	466
2142	12 hrs no RT				0.2	0.8	173	138		691	
2143	12 hrs no RT				0.09	0.69	51	35		389	
2144	12 hrs no RT				0.64	1.24	142	176		276	
2169	24 hrs + RT				0.08	0.68	91	62	157	774	839
2170	24 hrs + RT				0.79	1.39	167	232		294	
2171	24 hrs + RT				0.1	0.7	142	99		993	
2172	24 hrs + RT				0.18	0.78	298	233		1293	
2165	24 hrs no RT				0.19	0.79	264	209	166	1098	917
2166	24 hrs no RT				0.11	0.71	106	75		684	
2167	24 hrs no RT				0.34	0.94	182	171		504	
2168	24 hrs no RT				0.15	0.75	277	207		1383	
2181	48 hrs + RT				0.34	0.94	204	191	252	563	464
2182	48 hrs + RT				0.73	1.33	355	472		647	
2183	48 hrs + RT				0.46	1.06	211	224		487	
2184	48 hrs + RT				0.74	1.34	89	119		161	
2173	48 hrs no RT				0.34	0.94	174	164	138	482	480
2174	48 hrs no RT				0.24	0.84	197	166		691	
2175	48 hrs no RT				0.64	1.24	126	156		244	
2176	48 hrs no RT				0.13	0.73	90	66		505	
2153	1 wk + RT				0.53	1.13	56	64	85	120	133
2154	1 wk + RT				0.57	1.17	72	84		148	
2155	1 wk + RT				0.98	1.58	84	133		136	
2156	1 wk + RT				0.47	1.07	57	61		129	
2157	1 wk no RT				0.06	0.66	34	22	66	371	291
2158	1 wk no RT				0.21	0.81	133	108		514	
2159	1 wk no RT				0.54	1.14	93	106		196	
2160	1 wk no RT				0.32	0.92	29	27		83	
2149	2 wk + RT				0.2	0.8	53	43	72	214	327
2150	2 wk + RT				0.5	1.1	72	80		159	
2151	2 wk + RT				0.17	0.77	191	147		867	
2152	2 wk + RT				0.26	0.86	21	18		69	
2137	2 wk no RT				3.36	4.36	59	256	142	76	91
2138	2 wk no RT				0.73	1.33	50	66		91	
2139	2 wk no RT				1.12	2.12	46	98		87	
2140	2 wk no RT				1.35	2.35	63	149		110	

IN VIVO TNF INDUCTION

	1	2	3	4	5	6	7	8	9
A	1000		2161	6°	2185	12°	2169	24°	2181
B	500		2162	+ RT	2186	+	2170	+	2182
C	250		2163		2187	RT	2171	2172	2183
D	125		2164		2188		2172		2184
E	62		2177						
F	31°		2178	6°	2141	12°	2165	24°	2173
G	16°		2179	no RT	2142	no	2166	no	2174
H	0		2180		2143	RT	2167	RT	2175
					2144		2168		2176
	10	11	12	13	14	15	16	17	18
A	48°	2153	1wk	2149	2150	1702	Control	2081	control/2
B	+RT	2154	+	<u>2150</u>	RT	1698	Control	2145	2°
C		2155	RT	2151	RT	1724	Control	2146	2°
D		2156		2152	RT	2042	Control	2147	2°
E									
F	48°	2157	* WK	2137	2WK	2034	Control	2081	control
G	no RT	2158	* no	2138	no	2077	899-6	2145	
H		2159	RT	2139	RT	2078	891-6	2146	
		2160		2140		2080	control 1	2147	
	19								
A	10 ⁷ 6° +RT								
B	10 ⁷ 6° no RT								
C	10 ⁸ 6° +RT								
D	10 ⁸ 6° no RT								
E	10 ⁷ 24° +RT								
F	10 ⁷ 24° no RT								
G	10 ⁸ 24° +RT								
H	10 ⁸ 24° no RT								

not all supernatant assayed
he was spilled

* for these sets, there was insufficient supernatant to fill the second well

048 x (C43 1.84)

DATE: _____ TIME: _____
 NAME: _____
 ADDRESS: _____
 PHONE: _____

OPTIONAL DESIGN

1	2	3	4	5	6	7	8	9	10	11	12
		6° + RT		12° + RT		24° + RT		48° + RT		1WK + RT	
		6° - RT		12° - RT		24° no RT		48° - RT		1WK - RT	

READ MORE

~~invalid~~ = invalid data

OPTIONAL DESIGN

13	14	15	16	17	18	19	20
2WK + RT						10 ⁷	
						10 ⁸	
						10 ⁷	
						10 ⁸	
1WK no RT							

INDUCTION- in vivo							
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor Wt	Vol Buffer	[TNF]pg/ml
1702	Control	None		63	0.06	.6	0.37
1698	Control	None		67	0.07 2.0	1.0	1.03
1724	Control	None		2520	2.5 0.7	.6	0.42
2042	3616			528	0.6	.6	0.33
2034	3616+20+25			644	0.7	.6	0.41
2077	899-6			2090	2.9	1.0	0.47 0.89
2078	899-6			6186	4.5	1.0	0.44
2080	Control 1			938	0.9	.6	0.52
2081	Control 2			1124	1	1.0	1.31
2145	2 hrs + RT				0.51	.6	0.57
2146	2 hrs + RT				0.39	.6	0.49
2147	2 hrs + RT				0.06	.6	0.40

8

52
 .6
 512
 39.2

40ml 150 mM NaCl
 400 ml TRIS 1M pH 7.5
 400 ml EDTA 0.5M pH 7.5

protease inhibitors

/ DTT 1mM 40ul
 / PMSF 100mM 20ul
 casepstatin 5ug 4ul
 2 aprotinin 10ug 8ul

26 rows = 39 ml
 4.52

76
 2
 8152 wells

19 rows
 8
 152

INDUCTION- in vivo							wt. supernatant
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor Wt	Vol Buffer	[TNF] pg/ml
2161	6 hrs + RT				0.7	.6	.58
2162	6 hrs + RT				0.66	.6	0.47
2163	6 hrs + RT				0.68	.6	.53
2164	6 hrs + RT				0.28	.6	0.50
2177	6 hrs no RT				1.03	1.0	0.93
2178	6 hrs no RT				0.5	.6	1.45
2179	6 hrs no RT				0.23	.6	0.44 0.52
2180	6 hrs no RT				0.2	.6	0.45
2185	12 hrs + RT				0.5	.6	.55mg
2186	12 hrs + RT				0.54	.6	.500
2187	12 hrs + RT				0.47	.6	0.55
2188	12 hrs + RT				0.54	.6	0.52
2141	12 hrs no RT				0.19	.6	0.49
2142	12 hrs no RT				0.2	.6	.46
2143	12 hrs no RT				0.09	.6	0.42
2144	12 hrs no RT				0.64	.6	0.53
2169	24 hrs + RT				0.08	.6	0.57
2170	24 hrs + RT				0.79	.6	0.26
2171	24 hrs + RT				0.1	.6	0.38
2172	24 hrs + RT				0.18	.6	0.42
2165	24 hrs no RT				0.19	.6	0.56
2166	24 hrs no RT				0.11	.6	0.39
2167	24 hrs no RT				0.34	.6	0.51
2168	24 hrs no RT				0.15	.6	0.58
2181	48 hrs + RT				0.34	.6	0.56
2182	48 hrs + RT				0.73	.6	0.66
2183	48 hrs + RT				0.46	.6	0.61
2184	48 hrs + RT				0.74	.6	0.73
2173	48 hrs no RT				0.34	.6	0.52
2174	48 hrs no RT				0.24	.6	0.56
2175	48 hrs no RT				0.64	.6	0.73
2176	48 hrs no RT				0.13	.6	0.42
2153	1 wk + RT				0.53	.6	0.59
2154	1 wk + RT				0.57	.6	0.43
2155	1 wk + RT				0.98	.6	0.73
2156	1 wk + RT				0.47	.6	0.40
2157	1 wk no RT				0.06	.6	0.34
2158	1 wk no RT				0.21	.6	0.48
2159	1 wk no RT				0.54	.6	0.46
2160	1 wk no RT				0.32	.6	0.42
2149	2 wk + RT				0.2	.6	0.38
2150	2 wk + RT				0.5	.6	0.32
2151	2 wk + RT				0.17	.6	0.28
2152	2 wk + RT				0.26	.6	0.38
2137	2 wk no RT				3.36	1.0	1.25
2138	2 wk no RT				0.73	.6	0.58
2139	2 wk no RT				1.12	1.0	0.89
2140	2 wk no RT				1.35	1.0	0.77

600ul RIPA buffer added to tumors < 1 gm
 1000ul " " " " " 21 gm

spilled
 some
 supernat

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

#315B)

REQ NO 18784

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: SHIPMENTS ON A BASISREQUEST BY: Craig Sidley, MD DATE: 2-2-94REQUESTORS PHONE NUMBER: 2-0294AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 2-73731-5100PI: HallenVENDOR: FCRIPROTOCOL: 58671REQUESTED DELIVERY DATE: 2-0294PHONE: 2-0294SPECIES: MouseQUANTITY: 80STRAIN: Albino NudeSEX: M ☒ EITHERWEIGHT/AGE: 5-6 weeks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS:

PROCUREMENT DESK: 2-9364

HOUSE AT: CARLSON

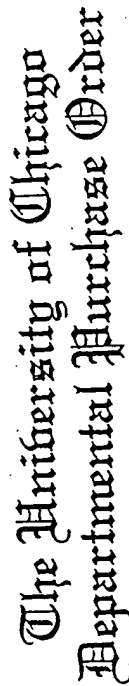
WYLER

☒ CLSC

FMI

OTHER

1863	AA108971	10^7 3616+RT	10.5	8	3
1864	AA108971	10^7 3616+RT	6	8	3.5
1865	AA108971	10^7 3616+RT	3	3	2
1866	AA108971	10^7 3616+RT	3	3.5	1.5
1883	AA108966	10^7 3616+RT	4.5	4	1
1884	AA108966	10^7 3616+RT	7.5	6	5
1886	AA108966	10^7 3616+RT	6	4	3
2074	AA108966	10^7 3616+RT	6.5	5	7
2032	AA111326	10^7 3616+RT	10	6	5.5
2033	AA111326	10^7 3616+RT	12	9	5.5
2035	AA111326	10^7 3616+RT	8.5	7.5	6
2072	AA111326	10^7 3616+RT	13	9.5	8
2036	AA111327	10^7 3616+RT	12	9	6
2037	AA111327	10^7 3616+RT	8.5	7	6
2039	AA111327	10^7 3616+RT	10	9	5
2073	AA111327	10^7 3616+RT	12.5	11.5	9
		10^7 3616+RT			
1871	AA108969	10^7 899-6+RT	6.5	7	4
1872	AA108969	10^7 899-6+RT	4.5	6.5	2.5
1873	AA108969	10^7 899-6+RT	8	8	3.5
1874	AA108969	10^7 899-6+RT	7	6	3
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	3.5	3	1
1881	AA108967	10^7 899-6+RT	6	4.5	2
1882	AA108967	10^7 899-6+RT	3	2.5	1
2044	AA111329	10^7 899-6+RT	9.5	8	5
2045	AA111329	10^7 899-6+RT	5.5	5	3
2046	AA111329	10^7 899-6+RT	9.5	8	5
2047	AA111329	10^7 899-6+RT	6	6	3.5
2048	AA111330	10^7 899-6+RT	7.5	6	4.5
2049	AA111330	10^7 899-6+RT	15	10	8
2050	AA111330	10^7 899-6+RT	7	6.5	4
2051	AA111330	10^7 899-6+RT	11	13	5
		10^7 899-6+RT			



THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

Department of Agriculture

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Vendor Name

Srstedt
 St James Church Rd
 Newton, N.C. 28658
 State _____ Zip Code _____
 Delivery charge? ☐ Yes ☒ No
 Payment terms _____
 Telephone No. 800-257-5101
 FAX No. _____

THE UNIVERSITY OF CHICAGO

Dept. Code: -

ANTHONY GREGORY MC-0085

Department: Spec Sup/ser 6-03

Room

Altin: 176037
State: 17 Zip Code: 60637

	Payroll No.
Authorized Signature _____	Extl. _____
Pfirt Name _____	
Account Code _____	Date _____

Order placed by phone? ☐ No ☒ Yes

Order placed with (name) Greer Sply Phone # 820-257-5101
 Order placed by (name) _____ Date _____

1987

7

DESCRIPTION	DATE	AMOUNT	REMARKS
...

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1000	65816 Dime stopper	19.22	
1	1000	55,518 13 ml centrifuge tubes	<u>57.92</u>	
			77.14	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

**BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER**

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0	Day 1	Day 2	weight	Day 3	Day 4	Day 5	Day 6	weight
1720	AA105283	10^7 899-6	25	17.5	14	26.5					
1710	AA105283	10^7 899-6	0	0	0	22.7					
2075	AA105283	10^7 899-6	10	9	3	20.7					
1713	AA105283	10^7 899-6	0	0	0	21.2					
2052	AA111331	10^7 899-6	14.5	12	8	20.3					
2053	AA111331	10^7 899-6	12	15.5	8	22.7					
2054	AA111331	10^7 899-6	14	15	6	19.7					
2055	AA111331	10^7 899-6	11	7	3.5	21.9					
2056	AA111332	10^7 899-6	12	15	7	24.5					
2057	AA111332	10^7 899-6	6	6	3	21.4					
2058	AA111332	10^7 899-6	12.5	11	6	23.9					
2076	AA111332	10^7 899-6	9.5	11	7	24.6					
2060	AA111333	10^7 899-6	14	17.5	4.5	25.5	12.5	12.5	8		23.7
2061	AA111333	10^7 899-6	11	10	5	21.6					
2062	AA111333	10^7 899-6	8	6	4	16.9					
2063	AA111333	10^7 899-6	9	8	6.5	21.0					
		10^7 899-6									
1697	AA104549	10^7 3616	14	17.5	9.5	29.3					
1708	AA104549	10^7 3616	0	0	0	21.9					
1709	AA104549	10^7 3616	0	0	0	27.3					
1723	AA104549	10^7 3616	0	0	0	26.3					
1877	AA108968	10^7 3616									
1878	AA108968	10^7 3616									
2041	AA111328	10^7 3616	6	5	3	22.3					
2043	AA111328	10^7 3616	13.5	12	7	25					
2064	AA111334	10^7 3616	8	6.5	4	23.1					
2065	AA111334	10^7 3616	16	16	9	23.3					
2066	AA111334	10^7 3616	12	12	6	23.2					
2067	AA111334	10^7 3616	8.5	6	3.5	14.5					
2068		10^7 3616	7	6	5	15.8					
2069		10^7 3616	10	10	7.5	23.2					
2070		10^7 3616	11	8	5	23.4					
2071		10^7 3616	11	10	5	24.0					
		10^7 3616									
1859	AA108972	RT Alone	9.5	10	3.5	23.9					
1860	AA108972	RT Alone	3/27/95								
1861	AA108972	RT Alone	12	13.5	7	21.5					
1862	AA108972	RT Alone	8	7	3	25.3					
1892	AA108964	RT Alone	7.5	8	5	22.8					
		RT Alone									
			12	13.5	7	21.5					

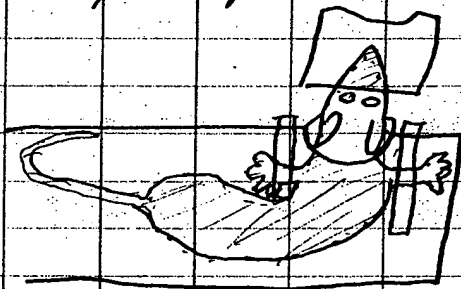
1863	AA108971	10^7 3616+RT	10	7	2.5	22.9
1864	AA108971	10^7 3616+RT	7.5	5	3	24.1
1865	AA108971	10^7 3616+RT	3.5	3	1	24.9
1866	AA108971	10^7 3616+RT	3	3	1	24.9
1883	AA108966	10^7 3616+RT	0	0	0	22
1884	AA108966	10^7 3616+RT	5.5	5.5	3	20.6
1886	AA108966	10^7 3616+RT	11.5	10	7	14.8
2074	AA108966	10^7 3616+RT	4	4	1	22
2032	AA111326	10^7 3616+RT	11.5	7.5	5	20.2
2033	AA111326	10^7 3616+RT	11.5	9	6	21.6
2035	AA111326	10^7 3616+RT	5.5	5.5	3	27.7
2072	AA111326	10^7 3616+RT	13	10	6	18.8
2036	AA111327	10^7 3616+RT	12	9	4	20.4
2037	AA111327	10^7 3616+RT	6	8	4	23.6
2039	AA111327	10^7 3616+RT	11	8	7.5	19.3
2073	AA111327	10^7 3616+RT	8.5	10	5	17.9
		10^7 3616+RT				
1871	AA108969	10^7 899-6+RT	7	7	3.5	23.3
1872	AA108969	10^7 899-6+RT	4.5	6	3	22.4
1873	AA108969	10^7 899-6+RT	7.5	8	4	22.4
1874	AA108969	10^7 899-6+RT	6.5	5	2.5	24.8
1879	AA108967	10^7 899-6+RT	0	0	0	26.0
1880	AA108967	10^7 899-6+RT	0	0	0	20.7
1881	AA108967	10^7 899-6+RT	0	0	0	24.4
1882	AA108967	10^7 899-6+RT	4	3	1	22.1
2044	AA111329	10^7 899-6+RT	9	7.5	5.5	20.9
2045	AA111329	10^7 899-6+RT	7	5	3	24.5
2046	AA111329	10^7 899-6+RT	8.5	7	5	22.7
2047	AA111329	10^7 899-6+RT	5	5	5	21.2
2048	AA111330	10^7 899-6+RT	5.5	8	3.5	19.2
2049	AA111330	10^7 899-6+RT	15	10.5	5	21.8
2050	AA111330	10^7 899-6+RT	6	6	4	21.4
2051	AA111330	10^7 899-6+RT	17.5	11	4.5	20.5
		10^7 899-6+RT				

TOLERANCE OF NUDE MICE TO WHOLE BRAIN RT

PURPOSE - In preparation for stereotactic brain protocol, need to determine maximum tolerated whole brain dose

PROCEDURE -

- ① Anesthetize mice w/ Ketamine/Xylazine 0.4 ml
- ② Tape front legs of mice with head protruding through lead shield & piece of lead over nose (eyes exposed)



- ③ Irradiate w/ Maxikon: Plexiglass-to-coll dist = 8 cm, 30 mA 150 KVP (see TLD measurements next page)

TLD @ surface of skull = 450-500 cGy
TLD under head = 325 cGy } 500 cGy intended

- ④ Pilot experiment = 3 mice

mouse #1 = $1000 \times 5 = 5000$ cGy
#2 = $500 \times 6 = 3000$ cGy
#3 = $20 \times 8 = 4000$ cGy

RESULTS -

mouse #1
mouse #2
mouse #3

RT dates
start end

status
died

TLO exprn Measmt on Meas Rn

Dr. Sidney C. Hyman Channel .035" TLD

100 KUP

35 mA

1-mm Al Filter

Ques. kya hai for b. + h. ok

Collier - do

Calculation TLD

500 kg, it has been quality,

11.30 ML

11. 2 4

12.33

11.38

11.59 ± .50

$$\left(\frac{7L}{D}\right)_{150kVn} = \frac{11.59 \text{ mC}}{5.00 \text{ Gy}} = 2.32 \frac{\mu\text{C}}{\text{Gy}}$$

TiD₂ on M₄C₁

11-54 MC

10. 42

49706

466 c6

1482064

② C. 11 N. C. 294

227 ✓ 256

CGA

L3256y7

3 11.10 48206

9.60, 414 60

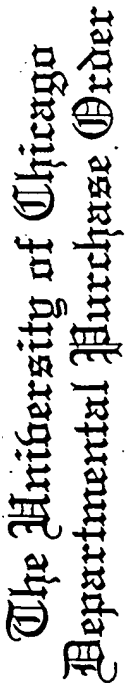
448

② Top
head
on nose

① Top-Hed-on unit

② Under must
must

Mouth

**Purchase Order Number**

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchaser; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Vendor Name

Fisher Scientific
1600 W Glenlake Ave.
Tosca, IL 60143

City State Zip Code
Payment Terms **Delivery charge?** ☐ Yes ☐ No
Telephone No. 2-3328 **FAX No.**

THE UNIVERSITY OF CHICAGO

U of C Rad Onc	Department	G-08	Room
Gneg	Sibbey		
5830 S. Ellis	MC 0085		
Chgo	1/L	60637	

Authorized Signature	Payroll No.
Print Name	Ext.
Account Code	Date

Order placed by phone? ☐ No ☒ Yes

Order placed by phone? ☐ No ☒ Yes

Order placed with (name)

Cindy

Order placed by (name)

Greg Sibbey

Phone #

2-0294

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
4	ea	Hamilton Syringes 10 ml 14-824-3	23.04	92.16
2	BOX	15 ml polypropylene tubes 14-959-70C	83.40	166.80
				258.48
				258.96
DEPARTMENT COPY			ORDER TOTAL	
			NOT VALID IF TOTAL EXCEEDS \$500.00	

NOT VALID IF TOTAL EXCEEDS \$500.00

**BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER**

DEPARTMENT COPY

ORDER TOTAL

Mouse	Treatment	1
-------	-----------	---

RR
2080
2081

Greg's Mouse Log

Greg's Mouse Log																	
Mouse #	Cage #	Group	Day 0			weight	Day 3										weight
1720	AA105283	10^7 899-6	20.5	28	16.5	29.3											
1710	AA105283	10^7 899-6	0	0	0	23.6											
2075	AA105283	10^7 899-6	12	12	7	23.4											
1713	AA105283	10^7 899-6	0	0	0	21.6											
2052	AA111331	10^7 899-6	16.5	14.5	12	21.4											
2053	AA111331	10^7 899-6	21	15	11.5	24.1											
2054	AA111331	10^7 899-6	16	12	2.5	22.0											
2055	AA111331	10^7 899-6	23.5	21	10.5	22.75	ec Red										
2056	AA111332	10^7 899-6	20	18	11	25.4											
2057	AA111332	10^7 899-6	9.5	8	6	21.8											
2058	AA111332	10^7 899-6	19	20	8.5	25.9											
2076	AA111332	10^7 899-6	15	16.5	9	27.5											
2060	AA111333	10^7 899-6	19.5	18	13	25.8											
2061	AA111333	10^7 899-6	20	16	11.5	26.2											
2062	AA111333	10^7 899-6	13.5	8	8.5	17.2											
2063	AA111333	10^7 899-6	17.5	13	10.5	23.5											
		10^7 899-6															
1697	AA104549	10^7 3616	18.5	18.5	13	25.2											
1708	AA104549	10^7 3616	0	0	0	21.4											
1709	AA104549	10^7 3616	0	0	6	25.7											
1723	AA104549	10^7 3616	0	0	0	26.0											
2069	AA108968	10^7 3616	12	15	8	24.8											
2070	AA108968	10^7 3616	13	11	6	26.2											
2041	AA111328	10^7 3616	8	8	5.5	23.6											
2043	AA111328	10^7 3616	22	17	11.5	26.5											
2068	AA111328	10^7 3616	5	4.5	3	14.9											
2071	AA111328	10^7 3616	15	12.5	8	26.3											
2064	AA111334	10^7 3616	8.5	8	5	24.1											
2065	AA111334	10^7 3616	23	24	14	25.8											
2066	AA111334	10^7 3616	16	15.5	6.5	25.2											
2067	AA111334	10^7 3616	8	6	4.5	12.4											
		10^7 3616															
1859	AA108972	RT Alone	9	9	4	25.5											
1861	AA108972	RT Alone	12	14.5	8	20.5											
1862	AA108972	RT Alone	6.5	8	5	24.9											
1892	AA108964	RT Alone	7	6.5	3.5	22.7											
		RT Alone															

[illegible]

[illegible]

Greg's Mouse Log

Greg's Mouse Log					
Mouse #	Cage #	Group			
1710	AA105283	10^7 899-6	6	0	0
2075	AA105283	10^7 899-6	16	14	8.5
1713	AA105283	10^7 899-6	0	0	0
2052	AA111331	10^7 899-6	23	17.5	13
2053	AA111331	10^7 899-6	18.5	12	13
2054	AA111331	10^7 899-6	20	14.5	13
2056	AA111332	10^7 899-6	21.5	15.5	12
2057	AA111332	10^7 899-6	9.5	11	6.5
2058	AA111332	10^7 899-6	19.5	24.5	10.5
2076	AA111332	10^7 899-6	17	18.5	9.5
2061	AA111333	10^7 899-6	23	15.5	12.5
2062	AA111333	10^7 899-6	15.5	"	10
2063	AA111333	10^7 899-6	20.5	16	11.5
		10^7 899-6			
1697	AA104549	10^7 3616	18.5	19	12
1708	AA104549	10^7 3616	0	0	0
1709	AA104549	10^7 3616	0	0	0
1723	AA104549	10^7 3616	0	0	0
2069	AA108968	10^7 3616	13.5	17	10
2070	AA108968	10^7 3616	13	16	17.5
2041	AA111328	10^7 3616	10.5	9.5	6.5
2068	AA111328	10^7 3616	4.5	4	2.5
2071	AA111328	10^7 3616	18	16.5	10
2064	AA111334	10^7 3616	9	10	7
2066	AA111334	10^7 3616	20	17	9
		10^7 3616			
1859	AA108972	RT Alone	10	10	4
1861	AA108972	RT Alone	12	13	10.5
1862	AA108972	RT Alone	6.5	7	4
1892	AA108964	RT Alone	7.5	7.5	3.5
		RT Alone			

~~22-3~~

1863	AA108971	10^7 3616+RT	9	6.5	4		24.2													
1864	AA108971	10^7 3616+RT	9	8.5	7		24.0													
1865	AA108971	10^7 3616+RT	2	2	1		24.1													
1866	AA108971	10^7 3616+RT	0	0	0		25.8													
1883	AA108966	10^7 3616+RT	0	0	0		21..8													
1884	AA108966	10^7 3616+RT	2	2	2		20.9													
1886	AA108966	10^7 3616+RT	0	0	0		21.8													
2074	AA108966	10^7 3616+RT	9	10	5.5		14.4													
2032	AA111326	10^7 3616+RT	9.5	7	5.5		20.9													
2033	AA111326	10^7 3616+RT	6	5	4		18.2													
2035	AA111326	10^7 3616+RT	13.5	12	6		18.5													
2072	AA111326	10^7 3616+RT	0	0	0		22.7													
2036	AA111327	10^7 3616+RT	10.5	9	4		20.7													
2037	AA111327	10^7 3616+RT	5	4	2.5		23.7													
2039	AA111327	10^7 3616+RT	8	7	4.5		18.6													
2073	AA111327	10^7 3616+RT	10	9	4.5		18.2													
		10^7 3616+RT																		
1871	AA108969	10^7 899-6+RT	6	5.5	2.5		19.4													
1872	AA108969	10^7 899-6+RT	4.5	6	4		23.5													
1873	AA108969	10^7 899-6+RT	7	2.5	2.5		24.0													
1874	AA108969	10^7 899-6+RT	3	2	1		26.1													
1879	AA108967	10^7 899-6+RT	0	0	0		27.2													
1880	AA108967	10^7 899-6+RT	0	0	0		20.7													
1881	AA108967	10^7 899-6+RT	0	0	0		25.2													
1882	AA108967	10^7 899-6+RT	0	0	0		23.7													
2044	AA111329	10^7 899-6+RT	4.5	5	3.5		23.2													
2045	AA111329	10^7 899-6+RT	0	0	0		25.8													
2046	AA111329	10^7 899-6+RT	6.5	5.5	4		23.7													
2047	AA111329	10^7 899-6+RT	0	0	0		22.5													
2048	AA111330	10^7 899-6+RT	3.5	3	2.5		18.0													
2049	AA111330	10^7 899-																		

Greg's Mouse Log

Greg's Mouse Log						
Mouse #	Cage #	Group				
1710	AA105283	10^7 899-6	0	0	0	23.7
2075	AA105283	10^7 899-6	17	19.5	9	24.1
1713	AA105283	10^7 899-6	0	0	0	21.5
2052	AA111331	10^7 899-6				
2053	AA111331	10^7 899-6				
2054	AA111331	10^7 899-6	11	15	8.5	19.4
2056	AA111332	10^7 899-6				
2057	AA111332	10^7 899-6	11.5	9	6.5	21.0
2058	AA111332	10^7 899-6				
2076	AA111332	10^7 899-6	20.5	17.5	13.5	28.4 sacked
2061	AA111333	10^7 899-6				
2062	AA111333	10^7 899-6	17	11	8.5	16.2 sacked face tumor
2063	AA111333	10^7 899-6	23	17.5	14.5	24.1 sacked
		10^7 899-6				
1697	AA104549	10^7 3616				
1708	AA104549	10^7 3616	0	0	0	20.2 sacked
1709	AA104549	10^7 3616	0	0	0	25.8
1723	AA104549	10^7 3616	0	0	0	25.4
2069	AA108968	10^7 3616	21	16.5	11	24.4
2070	AA108968	10^7 3616	20	16	10	22.2
2041	AA111328	10^7 3616	10	11	7.5	22.4 sacked
2068	AA111328	10^7 3616				died
2071	AA111328	10^7 3616	23	20.5	13	27.5
2064	AA111334	10^7 3616	13	12.5	7	24.5
2055	AA111334	10^7 3616	23	19	12	
		10^7 3616				
1859	AA108972	RT Alone	10.5	10	6	25.5
1861	AA108972	RT Alone	13.5	13	8	21.2
1862	AA108972	RT Alone	7	7	4	25.6
1892	AA108964	RT Alone	7	6.5	3	22.8
		RT Alone				

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

We placed our mice on Bactrim in Cummings Rm #1053 (on) due to the appearance of declining health and a number of deaths after tumor implantation. I apologize for not making you aware of this. Please continue Bactrim therapy for 1 week (until).

The mice placed on antibiotics include all mice with HALLAHAN/SIBLEY on the card (20 cages).

Cage #'s:

Sorry for the confusion.

Sincerely,

Gregory S. Sibley, M.D.

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	21.5	19.5	9					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	11	14	4.5					
2057	AA111332	10^7 899-6	13	10	7					
1709	AA104549	10^7 3616	0	0	0					
1723	AA104549	10^7 3616	0	0	0					
2069	AA108968	10^7 3616	23	20	13.5					
2070	AA108968	10^7 3616	23	19	11					
2064	AA111334	10^7 3616	15	14	7.5					
2041	"	10^7 3616	12	11	9					
1859	AA108972	RT Alone	11	10	6					
1861	AA108972	RT Alone	13.5	12.5	8					
1862	AA108972	RT Alone	6	6	3.5					
1892	AA108964	RT Alone	6	6	3.5					
1863	AA108971	10^7 3616+RT	8	6	4					
1864	AA108971	10^7 3616+RT	12	10	9					
1865	AA108971	10^7 3616+RT	0	0	0					
1866	AA108971	10^7 3616+RT	0	0	0					
1883	AA108966	10^7 3616+RT	0	0	0					
1884	AA108966	10^7 3616+RT	3	3.5	2					
1886	AA108966	10^7 3616+RT	0	0	0					
2074	AA108966	10^7 3616+RT	9	10	5					
2032	AA111326	10^7 3616+RT	6	5	5					
2033	AA111326	10^7 3616+RT	9	7	3.5					
2035	AA111326	10^7 3616+RT	8.9	7	6					
2072	AA111326	10^7 3616+RT	0	0	0					
2036	AA111327	10^7 3616+RT	10	9	4					
2037	AA111327	10^7 3616+RT	5	3.5	2.5					
2039	AA111327	10^7 3616+RT	7	8	3.5					
2073	AA111327	10^7 3616+RT	9.5	9	5					
1871	AA108969	10^7 899-6+RT	6.5	6.5	4					
1872	AA108969	10^7 899-6+RT	4	5	3.5					
1873	AA108969	10^7 899-6+RT	7	7	4.5					
1874	AA108969	10^7 899-6+RT	0	0	0					
1879	AA108967	10^7 899-6+RT	0		0					
1880	AA108967	10^7 899-6+RT	0		0					
1881	AA108967	10^7 899-6+RT	0		0					
1882	AA108967	10^7 899-6+RT	0		0					
2044	AA111329	10^7 899-6+RT	4.5	4	3.5					
2045	AA111329	10^7 899-6+RT	0	0	0					
2046	AA111329	10^7 899-6+RT	6.5	6	3					
2047	AA111329	10^7 899-6+RT	0	0	0					
2048	AA111330	10^7 899-6+RT	3	3.5	2					
2049	AA111330	10^7 899-6+RT	14	12	10					
2050	AA111330	10^7 899-6+RT	3	2.5	1					
2051	AA111330	10^7 899-6+RT	9	6.5	4.5					

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0	23.0				
2075	AA105283	10^7 899-6	21	18	6.5	21.9				
1713	AA105283	10^7 899-6	0	0	0	21.6				
2054	AA111331	10^7 899-6	11	15	4	19.2				
2057	AA111332	10^7 899-6	14.5	12	8.5	21.1				
1709	AA104549	10^7 3616	0	0	0	23.9				
1723	AA104549	10^7 3616	0	0	0	24.9				
2069	AA108968	10^7 3616								
2070	AA108968	10^7 3616								
2064	AA111334	10^7 3616	15.5	14	8	23.4				
2041		3616	13.5	12	9	21.9				
1859	AA108972	RT Alone	11.5	10	5	26.1				
1861	AA108972	RT Alone	6.5	6	4.5	24.9				
1862	AA108972	RT Alone	14	14	7.5	22.5				
1892	AA108964	RT Alone	6.5	6.5	3.5	22.8				
1863	AA108971	10^7 3616+RT	8	5	3	24.5				
1864	AA108971	10^7 3616+RT	12	11	8.5	25.6				
1865	AA108971	10^7 3616+RT	0	0	0	26.4				
1866	AA108971	10^7 3616+RT	0	0	0	25.8				
1883	AA108966	10^7 3616+RT	0	0	0	22.0				
1884	AA108966	10^7 3616+RT	3	3	2	22.3				
1886	AA108966	10^7 3616+RT	10	9	4.5	16.4				
2074	AA108966	10^7 3616+RT	0	0	0	21.5				
2032	AA111326	10^7 3616+RT	6	5	5	19.1				
2033	AA111326	10^7 3616+RT	6	5.5	3.5	22.4				
2035	AA111326	10^7 3616+RT	8	6	4	19.3				
2072	AA111326	10^7 3616+RT	0	0	0	22.7				
2036	AA111327	10^7 3616+RT	10	9	4	22.5				
2037	AA111327	10^7 3616+RT	4	5	2.5	24.0				
2039	AA111327	10^7 3616+RT	8	8	5	12.4				
2073	AA111327	10^7 3616+RT	8	9	5	19.4				
1871	AA108969	10^7 899-6+RT	5	3	2	25.4				
1872	AA108969	10^7 899-6+RT	6.5	5.5	3	22.6				
1873	AA108969	10^7 899-6+RT	7	7.5	5	23.3				
1874	AA108969	10^7 899-6+RT	0	0	0	26.1				
1879	AA108967	10^7 899-6+RT	0	0	0	28.7				
1880	AA108967	10^7 899-6+RT	0	0	0	21.9				
1881	AA108967	10^7 899-6+RT	0	0	0	25.6				
1882	AA108967	10^7 899-6+RT	0	0	0	23.6				
2044	AA111329	10^7 899-6+RT	4	4	3.5	22.8				
2045	AA111329	10^7 899-6+RT	0	0	0	25.6				
2046	AA111329	10^7 899-6+RT	0.5	6	3	23.7				
2047	AA111329	10^7 899-6+RT	0	0	0	21.7				
2048	AA111330	10^7 899-6+RT	4	3	2	18.3				
2049	AA111330	10^7 899-6+RT	14	13	6	22.6				
2050	AA111330	10^7 899-6+RT	3	3	2	22.7				
2051	AA111330	10^7 899-6+RT	8	10	3.5	21.7				

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	φ							
2075	AA105283	10^7 899-6	20	15.5	6					
1713	AA105283	10^7 899-6	φ							
2054	AA111331	10^7 899-6	14	9.5	4					
2057	AA111332	10^7 899-6	15	11.5	8					
1709	AA104549	10^7 3616	φ							
1723	AA104549	10^7 3616	φ							
2069	AA108968	10^7 3616								
2070	AA108968	10^7 3616								
2064	AA111334	10^7 3616	15.5	13.5	10.5					
2041	AA111334	10^7 3616	14	12	9					
1859 φ	AA108972	RT Alone	11	9.5	7					
1861 R	AA108972	RT Alone face	11	9.5	7	14.5	13	12.5		
1862 LR	AA108972	RT Alone	5.5	5	3.5					
1892 (L)	AA108964	RT Alone <small>noxic tail</small>	6.5	6	3.5					
1863 φ	AA108971	10^7 3616+RT	6	5	4					
1864 L	AA108971	10^7 3616+RT	11	10	9.5					
1865 R	AA108971	10^7 3616+RT	φ							
1866 LR	AA108971	10^7 3616+RT	φ							
1883 φ	AA108966	10^7 3616+RT	φ							
1884 L	AA108966	10^7 3616+RT	2	2	2					
1886 LR	AA108966	10^7 3616+RT	φ							
2074 R	AA108966	10^7 3616+RT	8.5	10	5					
2032 φ	AA111326	10^7 3616+RT	4.5	5	4.5					
2033 L	AA111326	10^7 3616+RT	5.5	5	3					
2035 LR	AA111326	10^7 3616+RT	8	7	5					
2072 R	AA111326	10^7 3616+RT	φ							
2036 φ	AA111327	10^7 3616+RT	8.5	8	5					
2037 L	AA111327	10^7 3616+RT	4.5	4	2.5					
2039 LR	AA111327	10^7 3616+RT	8	7	6					
2073 R	AA111327	10^7 3616+RT	6.5	7	3					
1871 φ	AA108969	10^7 899-6+RT	φ							
1872 L	AA108969	10^7 899-6+RT	2.5	2.5	2					
1873 R	AA108969	10^7 899-6+RT	7	7.5	6					
1874 LR	AA108969	10^7 899-6+RT	φ							
1879	AA108967	10^7 899-6+RT								
1880 L	AA108967	10^7 899-6+RT	φ							
1881 R	AA108967	10^7 899-6+RT	φ							
1882 LR	AA108967	10^7 899-6+RT	φ							
2044 φ	AA111329	10^7 899-6+RT	4.5	4	2.5					
2045 L	AA111329	10^7 899-6+RT	φ							
2046 R	AA111329	10^7 899-6+RT	6.5	6	3					
2047 LR	AA111329	10^7 899-6+RT	φ							
2048 φ	AA111330	10^7 899-6+RT	3	3	2					
2049 L	AA111330	10^7 899-6+RT	10	9	6	13	12	10		
2050 R	AA111330	10^7 899-6+RT	10	9	6					
2051 LR	AA111330	10^7 899-6+RT	10	9	6					

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0	23.3				
2075	AA105283	10^7 899-6	21	17	8	22.4				
1713	AA105283	10^7 899-6	0	0	0	21.4				
2054	AA111331	10^7 899-6	13.5	6.5	5	20.5				
2057	AA111332	10^7 899-6	18.5	16.5	14.5	23.3				
1709	AA104549	10^7 3616	0	0	0	24.0				
1723	AA104549	10^7 3616	0	0	0	23.5				
2041	AA111334	10^7 3616	18	16	14	24.8				
2064	AA111334	10^7 3616	18.5	16.5	12	23.5				
1859	AA108972	RT Alone	12	9.5	8.5	26.1				
1861	AA108972	RT Alone <i>9ac</i>								
1862	AA108972	RT Alone	5.5	4.5	3.5	25.1				
1892	AA108964	RT Alone	5.5	5.5	3.5	24.1				
1863	AA108971	10^7 3616+RT	5	4.5	3.5	25.0				
1864	AA108971	10^7 3616+RT	11	14	11	25.1				
1865	AA108971	10^7 3616+RT	0	0	0	25.9				
1866	AA108971	10^7 3616+RT	0	0	0	25.3				
1883	AA108966	10^7 3616+RT	0	0	0	22.2				
1884	AA108966	10^7 3616+RT	2	2	1	24.4				
1886	AA108966	10^7 3616+RT	0	0	0	21.3				
2074	AA108966	10^7 3616+RT	8	9	6	16.3				
2032	AA111326	10^7 3616+RT	4.5	5.5	3.5	22.0				
2033	AA111326	10^7 3616+RT	5.5	4.5	3.5	24.8				
2035	AA111326	10^7 3616+RT	8	6.5	5	21.7				
2072	AA111326	10^7 3616+RT	0	0	0	24.2				
2036	AA111327	10^7 3616+RT	8	7	4	23.9				
2037	AA111327	10^7 3616+RT	4	4.5	1.5	24.6				
2039	AA111327	10^7 3616+RT	9	9	8	20.3				
2073	AA111327	10^7 3616+RT	7	7	4	20.2				
1871	AA108969	10^7 899-6+RT	5	5	3	23.3				
1872	AA108969	10^7 899-6+RT <i>950/45</i>								
1873	AA108969	10^7 899-6+RT	6.5	8	5.5	23.8				
1874	AA108969	10^7 899-6+RT	0	0	0	26.1				
1880	AA108967	10^7 899-6+RT	0	0	0	22.4				
1881	AA108967	10^7 899-6+RT	0	0	0	25.4				
1882	AA108967	10^7 899-6+RT	0	0	0	29.4				
2044	AA111329	10^7 899-6+RT	3.5	3.5	3	23.3				
2045	AA111329	10^7 899-6+RT	0	0	0	25.9				
2046	AA111329	10^7 899-6+RT	5.5	6	2.5	24.8				
2047	AA111329	10^7 899-6+RT	0	0	0	22.1				
2048	AA111330	10^7 899-6+RT	2.5	2.0	1	17.4				
2049	AA111330	10^7 899-6+RT	13.5	11	9	24.7				
2050	AA111330	10^7 899-6+RT	4	3	2	23.2				
2051	AA111330	10^7 899-6+RT	8.5	8.0	6.5	22.9				

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	-16	12	3					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	11	5	3.5					
2057	AA111332	10^7 899-6	-20	12.5	15					
1709	AA104549	10^7 3616	0	0	0					
1723	AA104549	10^7 3616	0	0	0					
2041	AA111334	10^7 3616	-19	17	15					
2064	AA111334	10^7 3616	12.5	17	13.5					
1859	AA108972	RT Alone	-12	11	8					
1861	AA108972	RT Alone								
1862	AA108972	RT Alone	-5	4	3.5					
1892	AA108964	RT Alone	-6	5	4					
1863	AA108971	10^7 3616+RT	5	5	3.5					
1864	AA108971	10^7 3616+RT	-13	4.5	5.5					
1865	AA108971	10^7 3616+RT	0	0	0					
1866	AA108971	10^7 3616+RT	-0	0	0					
1883	AA108966	10^7 3616+RT	-0	0	0					
1884	AA108966	10^7 3616+RT	2	2.5	1					
1886	AA108966	10^7 3616+RT	0	0	0					
2074	AA108966	10^7 3616+RT	-9.5	8.5	5					
2032	AA111326	10^7 3616+RT	4.5	4	3.5					
2033	AA111326	10^7 3616+RT	-5.5	5	3					
2035	AA111326	10^7 3616+RT	-8	6.5	4.5					
2072	AA111326	10^7 3616+RT	-0	0	0					
2036	AA111327	10^7 3616+RT	8	7	4					
2037	AA111327	10^7 3616+RT	4	4	2					
2039	AA111327	10^7 3616+RT	9.5	8	6					
2073	AA111327	10^7 3616+RT	7	7	3					
1871	AA108969	10^7 899-6+RT	-5.5	5	3					
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	2.5	6	5					
1874	AA108969	10^7 899-6+RT	0	0	0					
1880	AA108967	10^7 899-6+RT	-0	0	0					
1881	AA108967	10^7 899-6+RT	0	0	0					
1882	AA108967	10^7 899-6+RT	0	0	0					
2044	AA111329	10^7 899-6+RT	-4	3.5	3					
2045	AA111329	10^7 899-6+RT	0	0	0					
2046	AA111329	10^7 899-6+RT	-5	5	2.5					
2047	AA111329	10^7 899-6+RT								
2048	AA111330	10^7 899-6+RT	2	3	1.5					
2049	AA111330	10^7 899-6+RT	16	13	11					
2050	AA111330	10^7 899-6+RT	2	3	2					
2051	AA111330	10^7 899-6+RT	9	8	5.5					

518
 17
 126
 18

306
 13
 918

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0	24.8				
2075	AA105283	10^7 899-6	0	0	0	21.8				
1713	AA105283	10^7 899-6	3	2	1	21.8				
2054	AA111331	10^7 899-6	0	0	0	21.8				
2057	AA111332	10^7 899-6								
1709	AA104549	10^7 3616								
1723	AA104549	10^7 3616	-0	0	0	23.7				
2041	AA111334	10^7 3616								
2064	AA111334	10^7 3616								
1859	AA108972	RT Alone	-13	11.5	8	23.9				
1861	AA108972	RT Alone								
1862	AA108972	RT Alone	-4.5	5	4	25.8				
1892	AA108964	RT Alone	5	5.5	4	24.3				
1863	AA108971	10^7 3616+RT	-4.5	4	3	24.6				
1864	AA108971	10^7 3616+RT	+13	10.5	7.5	26.3				
1865	AA108971	10^7 3616+RT	+2.5	2.5	1	26.5				
1866	AA108971	10^7 3616+RT	+3	3.5	1	25.8				
1883	AA108966	10^7 3616+RT	+0	0	0	22.6				
1884	AA108966	10^7 3616+RT	-1.5	1.5	1.5	24.3				
1886	AA108966	10^7 3616+RT	-0	0	0	21.4				
2074	AA108966	10^7 3616+RT	-9.5	8	6	17.3				
2032	AA111326	10^7 3616+RT	+7	5	4	21.4				
2033	AA111326	10^7 3616+RT	-4.5	4	3	24.3				
2035	AA111326	10^7 3616+RT	-8	7	5.5	21.4				
2072	AA111326	10^7 3616+RT	0	0	0	23.5				
2036	AA111327	10^7 3616+RT	+7	7	3	22.9				
2037	AA111327	10^7 3616+RT	+3.5	3	2	24.7				
2039	AA111327	10^7 3616+RT	+9	8	6.5	18.3				
2073	AA111327	10^7 3616+RT	-6	5.5	2	19.5				
1871	AA108969	10^7 899-6+RT	-4	4	3	23.7				
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	6.5	6.5	3	23.6				
1874	AA108969	10^7 899-6+RT	0	0	0					
1880	AA108967	10^7 899-6+RT	-0	0	0	21.8				
1881	AA108967	10^7 899-6+RT	0	0	0	26.6				
1882	AA108967	10^7 899-6+RT	-0	0	0	23.5				
2044	AA111329	10^7 899-6+RT	-4	3.5	2.5	22.9				
2045	AA111329	10^7 899-6+RT	+0	0	0	25.9				
2046	AA111329	10^7 899-6+RT	+4.5	4	3	23.8				
2047	AA111329	10^7 899-6+RT								
2048	AA111330	10^7 899-6+RT	-3.5	3	1.5	16.8				
2049	AA111330	10^7 899-6+RT	-15.5	13.5	11	26.4				
2050	AA111330	10^7 899-6+RT	-2.5	2	1	23.2				
2051	AA111330	10^7 899-6+RT	-9	7.5	5	22.6				

Apoptosis Assay

Purpose - To determine whether apoptosis plays a role in V-87 cell killing with TNF, RT, R3616 virus or R899-6 virus or combinations of these

Methods - 8 T150 flasks of V87 cells were grown to ~80% confluence.

	7 ^{am}	1 ^{pm}	5 ^{pm}	1 ^{pm}
	-6°	-4°	-2°	0° 2° 4° → 24°
Control (no Rx)				Harvest
TNF (100 ng/ml)			TNF	Harvest
RT (20 Gy) + TNF			TNF	Harvest
RT (20 Gy)			RT	Harvest
3.8 ml R3616 (10 ⁷ PFU) + RT	infect		RT	Harvest
R3616 (10 ⁷ PFU)	infect			Harvest
2.6 ml R899-6 (10 ⁷ PFU) + RT	infect		RT	Harvest
R899-6 (10 ⁷ PFU)	infect			

1. Virally infected cells flasks were ~~at~~ treated with 10⁷ PFU of virus (moi approx 0.5) in 6 ml 199V media. Other flasks ^{incubated} received 6 ml of 199V ~~5~~ virus as control.
2. At 2 hrs, an additional ¹⁴ ml of 10% FCS media was added to bring total volume to ~~30~~ 20 cc. (Actually I added 10% FCS by mistake x 3 hrs, switched to 0% FCS @ 12^h, this required decanting media from infected flasks)
3. Add TNF to appropriate flasks = .01 mg/ml stock = .01 ^{ng} /ul add 10 ul (100 ng) to 20 ml

APOPTOSIS ASSAY

<u>Sample</u>	<u>Apoptosis (%)</u>
Baseline	2.3
Positive Control	80.4
No Treatment (-TdT)	0.3
No Treatment	1.9
RT 20 Gy	19.3
TNF	2.6
TNF + RT	19
R3616 (MOI=0.5)	29.3
R3616 + RT	23.2
R899-6 (MOI=0.5)	26.8
R899-6 + RT	11.4

U87

Neg. control
No TdT

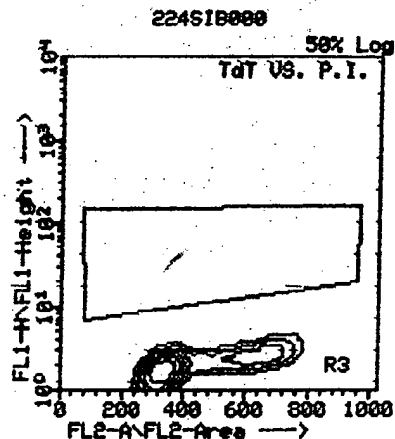
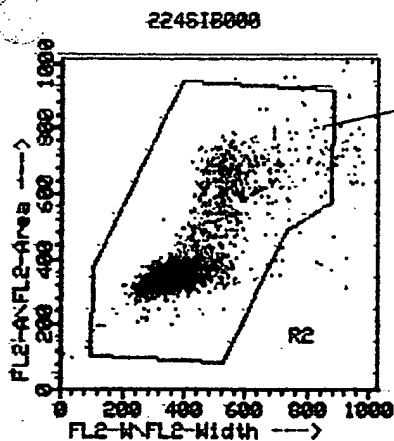
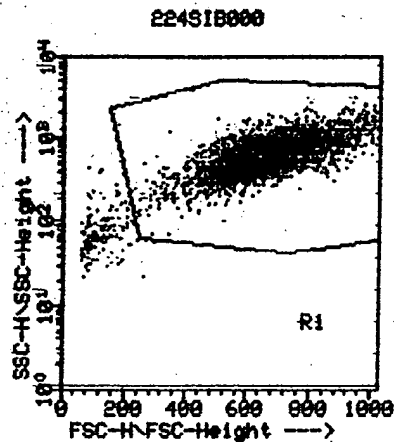
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 14:54:28

SELECTED PREFERENCES: Arithmetic/Linear

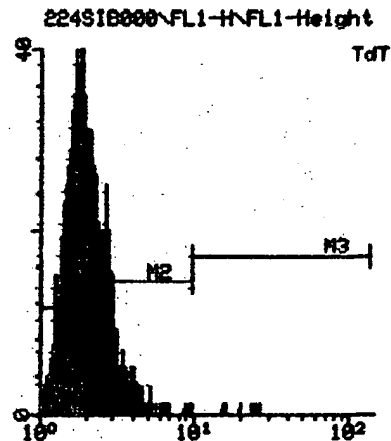


224SIB000

Region Stats

File: 224SIB000 Sample: GREG SIBLE
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 2368

Rgn	Events	% Gated	% Total
1 R1	2368	100.00	78.93
2 R2	2368	100.00	78.93
3 R3	8	0.34	0.27



224SIB000-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 224SIB000

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2368	100.00	43	1.91
1	1.00, 2.01	2108	89.02	43	1.04
2	2.01, 9.02	273	11.53	20	3.16
3	9.02, 136	7	0.30	1	19.01

CONTROL

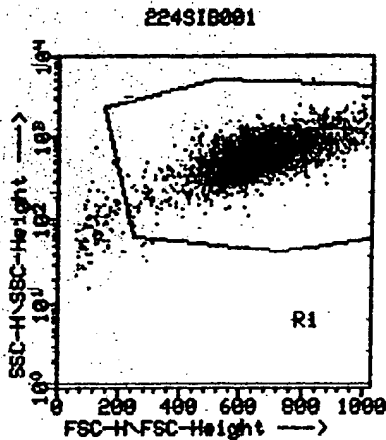
BECTON
DICKINSON

LVSYS II Ver 1.1

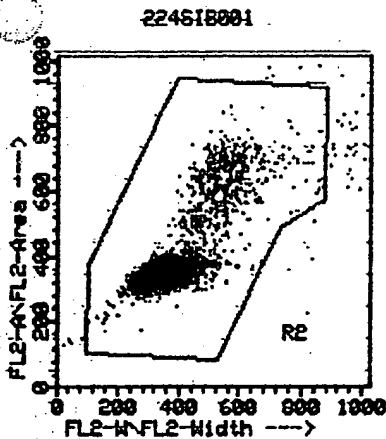
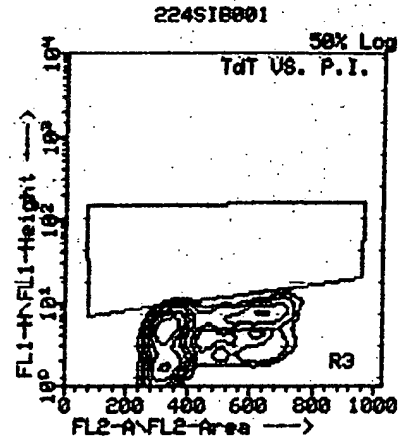
DATE:

TIME: 14:56:24

SELECTED PREFERENCES: Arithmetic/Linear



*serum
depleted*



224SIB001

Region Stats

File: 224SIB001 Sample: GREG SIBLE

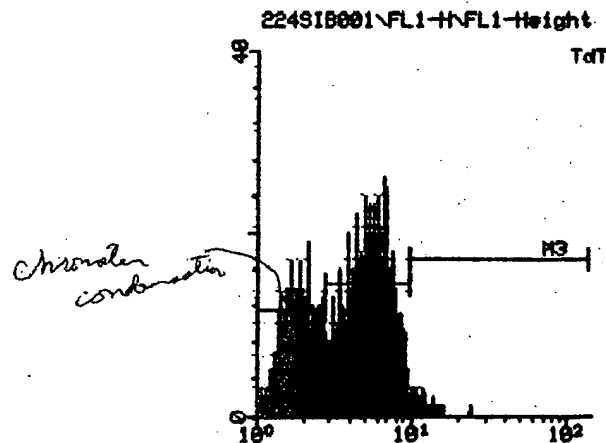
Date: Gate G2= R1ANDR2

Selected Preferences Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2514

Rgn	Events	% Gated	% Total
1 R1	2514	100.00	83.80
2 R2	2514	100.00	83.80
3 R3	13	0.52	0.43



224SIB001\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 224SIB001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2514	100.00	26	4.33
1	1.00, 2.01	819	32.58	19	1.91
2	2.01, 9.82	1657	65.91	26	5.33
3	9.82, 136	48	1.91	3	11.09

RT

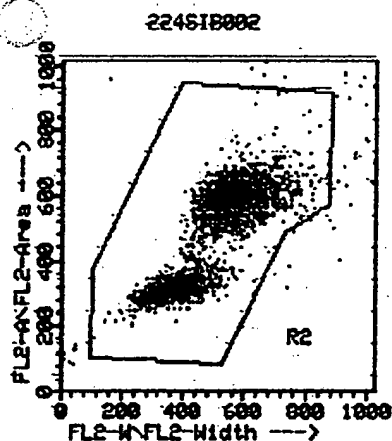
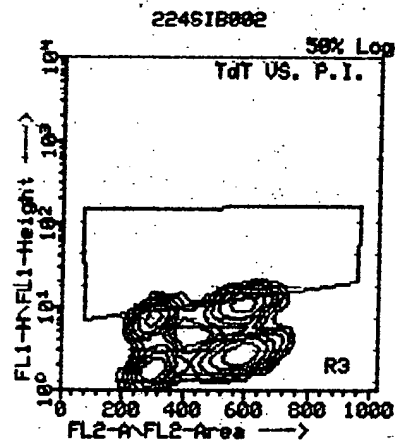
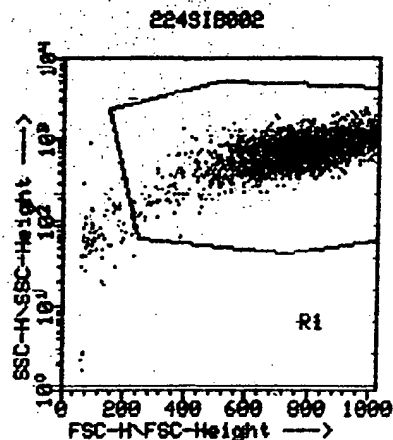
**BECTON
DICKINSON**

LYSYS II Ver 1.1

DATE:

TIME: 14:58:36

SELECTED PREFERENCES: Arithmetic/Linear



224SIB002

Region Stats

File: 224SIB002 Sample: GREG SIBLE

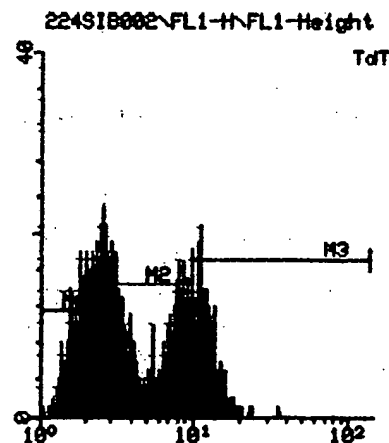
Date: Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2343

Rgn	Events	% Gated	% Total
1 R1	2343	100.00	78.10
2 R2	2343	100.00	78.10
3 R3	209	8.92	6.97



224SIB002-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 224SIB002

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2343	100.00	23	3.34
1	1.00, 2.81	937	39.99	23	2.15
2	2.81, 9.82	974	41.57	19	5.03
3	9.82, 136	451	19.25	21	11.06

TNF

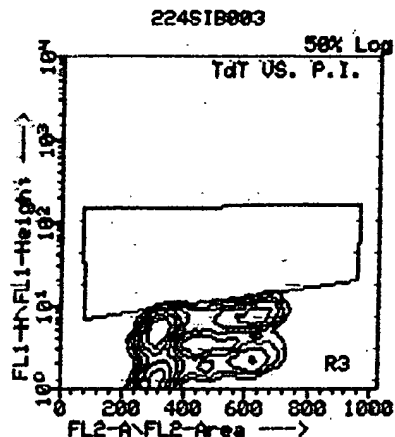
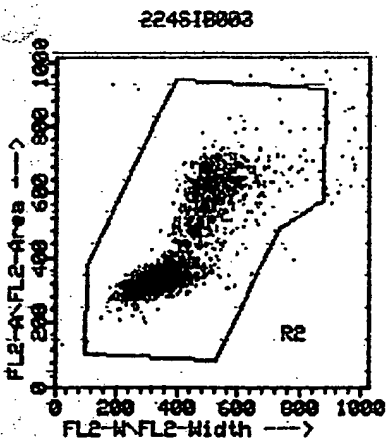
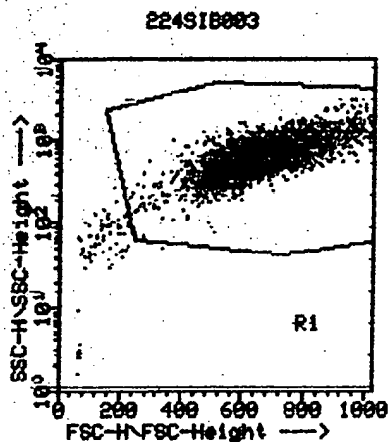
**BECTON
DICKINSON**

LYSYS II Ver 1.1

DATE:

TIME: 15:00:12

SELECTED PREFERENCES: Arithmetic/Linear



224SIB003

Region Stats

File: 224SIB003 Sample: GREG SIBLE

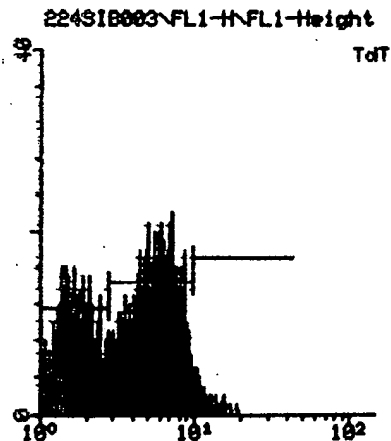
Date: Gate G2= R1ANDR2

Selected Preference: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2533

Rgn	Events	% Gated	% Total
1 R1	2533	100.00	84.43
2 R2	2533	100.00	84.43
3 R3	17	0.67	0.57



224SIB003\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 224SIB003

Selected Preference: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9918	2533	100.00	29	4.14
1	1.00, 2.81	953	37.62	29	1.65
2	2.81, 9.82	1522	60.09	22	5.57
3	9.82, 136	66	2.61	5	11.09

TNF+RT

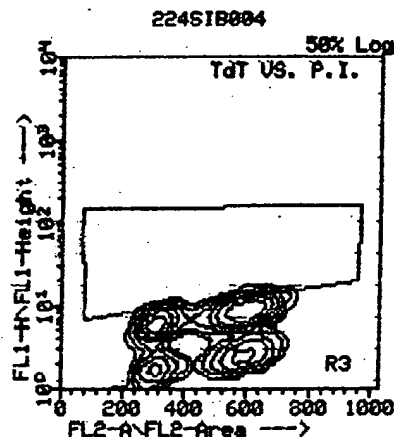
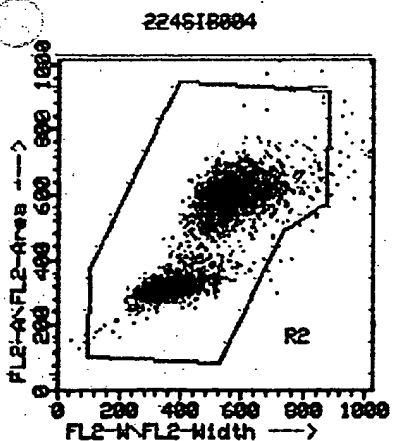
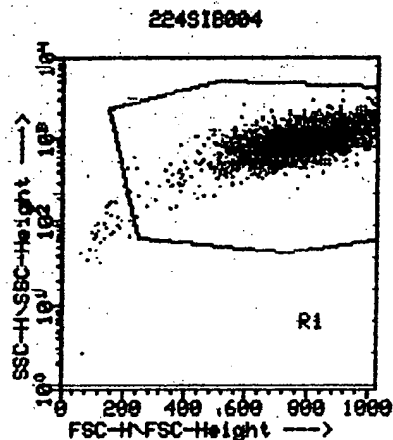
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:01:05

SELECTED PREFERENCES: Arithmetic/Linear



224SIB004

----- Region Stats -----

File: 224SIB004 Sample: GREG SIBLE

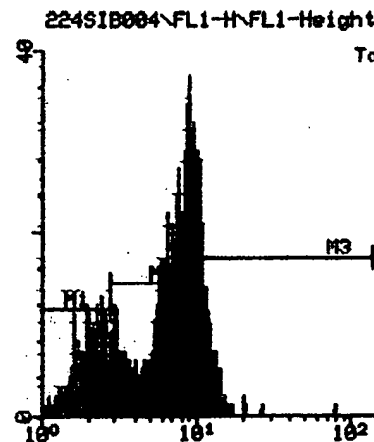
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2530

Rgn	Events	% Gated	% Total
1 R1	2530	100.00	84.33
2 R2	2530	100.00	84.33
3 R3	61	2.41	2.03



224SIB004\FL1-H-FL1-Height

----- Arithmetic Histogram Statistics for 224SIB004 -----

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9918	2530	100.00	37	7.17
1	1.00, 2.01	511	20.20	13	2.07
2	2.01, 9.02	1571	62.09	37	7.30
3	9.02, 136	401	19.01	29	10.75

3616

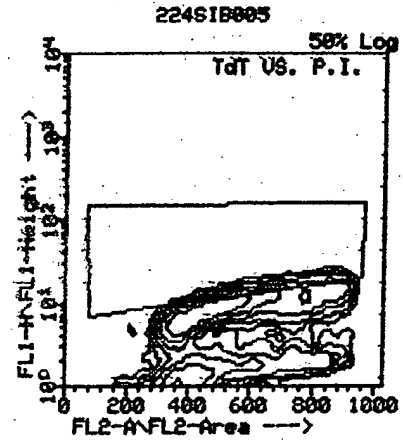
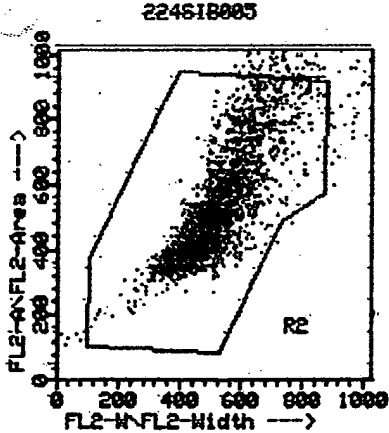
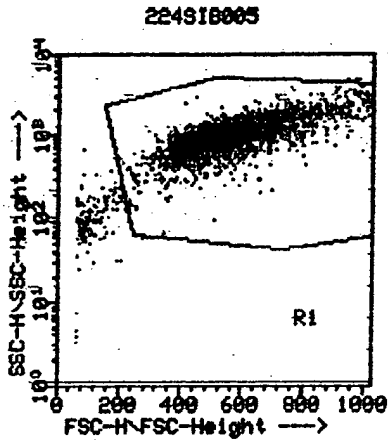
**BECTON
DICKINSON**

LYSYS II Ver 1.1

DATE:

TIME: 15:01:58

SELECTED PREFERENCES: Arithmetic/Linear



224SIB005

----- Region Stats -----

File: 224SIB005 Sample: GREG SIBLE

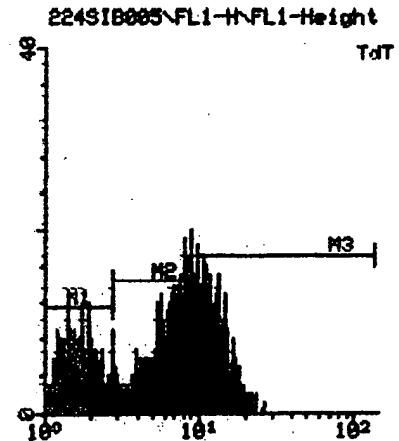
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2002

Rgn	Events	% Gated	% Total
1 R1	2002	100.00	66.73
2 R2	2002	100.00	66.73
3 R3	189	9.44	6.30



224SIB005-FL1-H-FL1-Height

----- Arithmetic Histogram Statistics for 224SIB005 -----

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2002	100.00	20	7.17
1	1.00, 2.01	524	26.17	20	1.61
2	2.01, 9.02	909	45.40	20	6.98
3	9.02, 136	567	29.32	17	12.30

3616 + RT

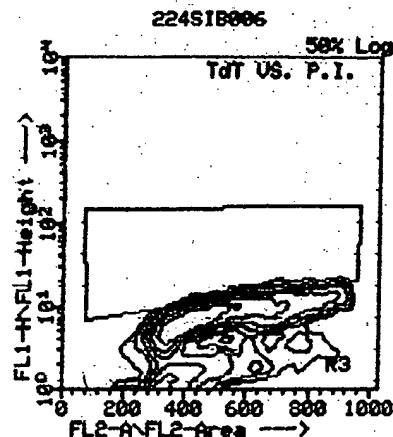
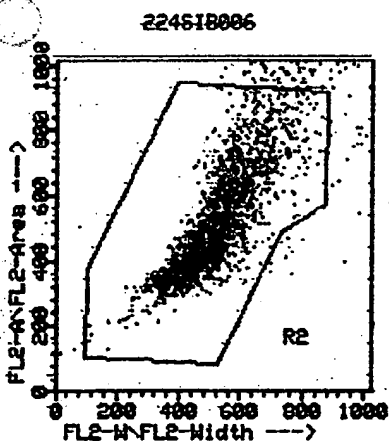
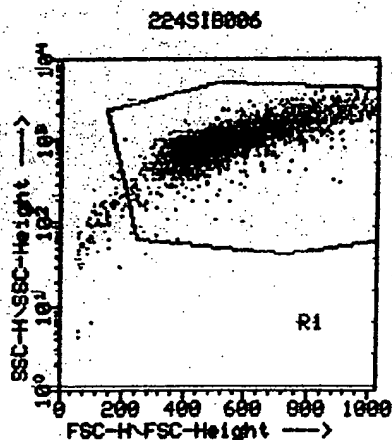
**BECTON
DICKINSON**

LYSYS II Ver 1.1

DATE:

TIME: 15:02:49

SELECTED PREFERENCES: Arithmetic/Linear



224SIB006

Region Stats

File: 224SIB006 Sample: GREG SIBLE

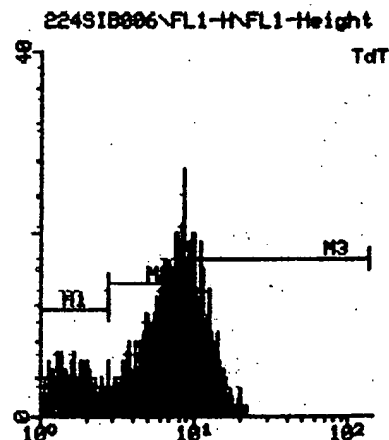
Date: Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2017

Rgn	Events	% Gated	% Total
1 R1	2017	100.00	67.23
2 R2	2017	100.00	67.23
3 R3	74	3.67	2.47



224SIB006 FL1-H FL1-Height

Arithmetic Histogram Statistics for 224SIB006

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2017	100.00	27	7.04
1	1.00, 2.01	343	17.01	13	1.65
2	2.01, 9.02	1227	60.83	27	6.79
3	9.02, 136	467	23.15	20	11.65

899-6

BECTON
DICKINSON

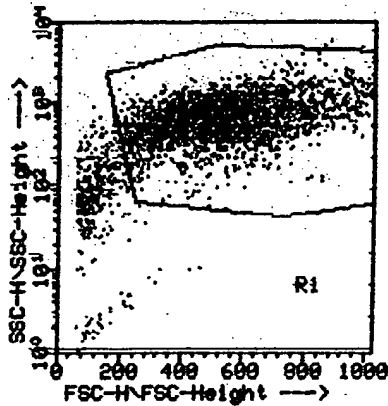
LYSYS II Ver 1.1

DATE:

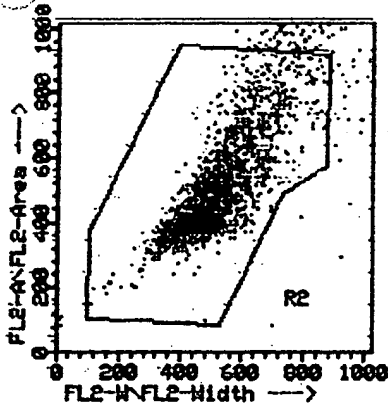
TIME: 15:03:40

SELECTED PREFERENCES: Arithmetic/Linear

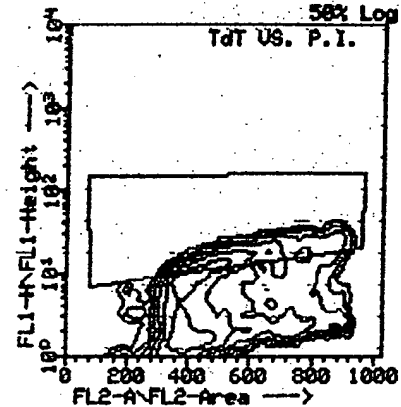
224SIB007



224SIB007



224SIB007

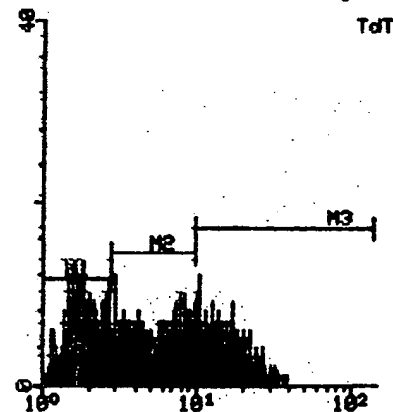


224SIB007

----- Region Stats -----
 File: 224SIB007 Sample: OREG SIBL
 Date: Gate G2= R1ANDR2
 Selected Preferences: Arithmetic/Line
 Parameters: FL2-A(LIN), FL1-H(LOG)
 Total= 3000 Gated= 1710

Rgn	Events	% Gated	% Total
1 R1	1710	100.00	57.00
2 R2	1710	100.00	57.00
3 R3	366	21.40	12.20

224SIB007/FL1-H/FL1-Height



224SIB007/FL1-H/FL1-Height

----- Arithmetic Histogram Statistics for 224SIB007
 Selected Preferences: Arithmetic/Linear
 Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1710	100.00	13	4.49
1	1.00, 2.01	599	35.03	13	1.04
2	2.01, 9.02	658	38.48	12	5.33
3	9.02, 136	459	26.04	12	14.20

899-6 + RT

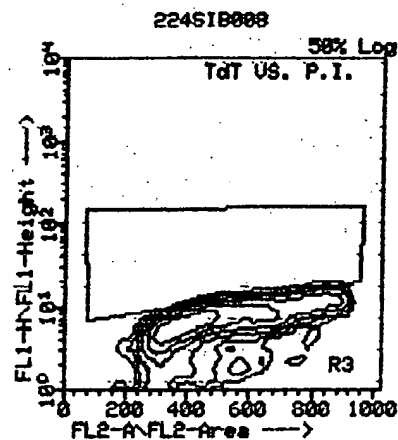
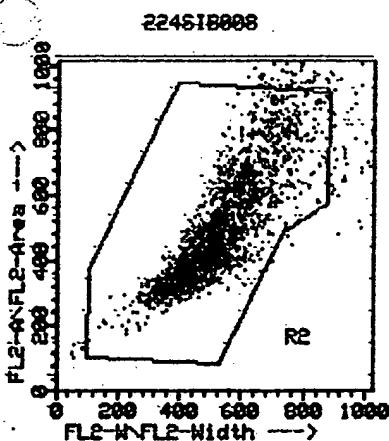
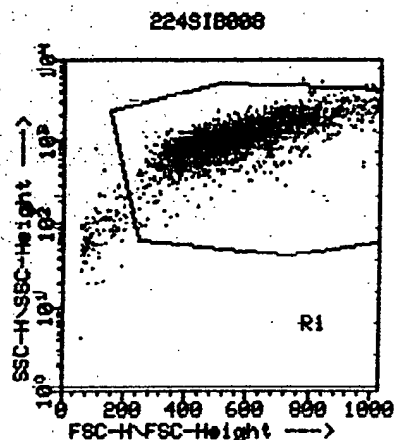
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LYSYS II Ver 1.1

DATE:

TIME: 15:04:34

SELECTED PREFERENCES: Arithmetic/Linear



224SIB008

----- Region Stats -----

File: 224SIB008 Sample: GREG SIBLE

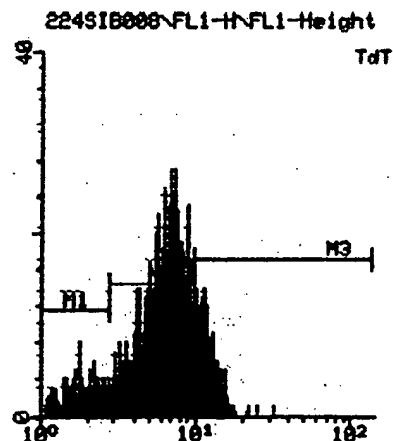
Date: Gate G2= RIANDR2

Selected Preferences: Arithmetic/Line

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2068

Rgn	Events	% Gated	% Total
1 R1	2068	100.00	68.93
2 R2	2068	100.00	68.93
3 R3	30	1.45	1.00



224SIB008\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB008

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2068	100.00	27	6.73
1	1.00, 2.01	218	10.54	8	1.04
2	2.01, 9.02	1515	73.26	27	6.49
3	9.02, 136	349	16.00	14	11.44

BECTON
DICKINSON

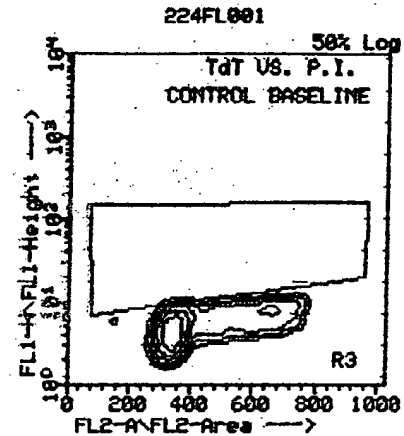
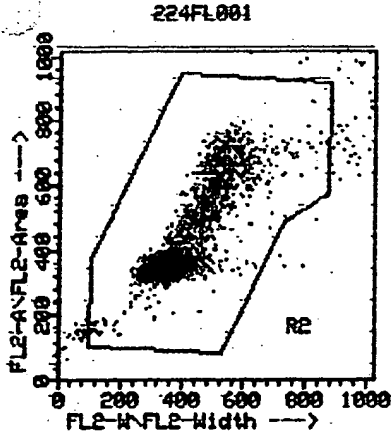
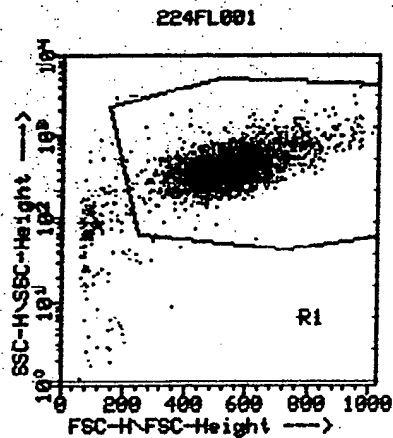
LYSYS II Ver 1.1

Baseline for
Positive Control

DATE:

TIME: 15:11:28

SELECTED PREFERENCES: Arithmetic/Linear



224FL001

----- Region Stats -----

File: 224FL001 Sample: FL5.12 CONTI

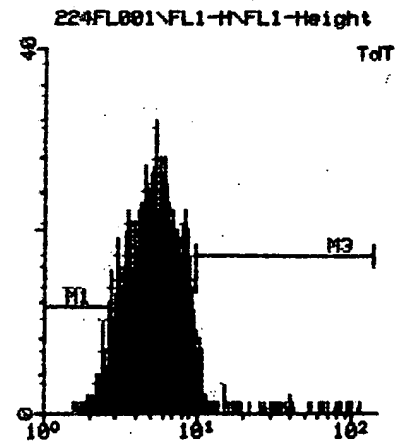
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Line

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2487

Rgn	Events	% Gated	% Total
1 R1	2487	100.00	82.90
2 R2	2487	100.00	82.90
3 R3	58	2.33	1.93



224FL001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224FL001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9918	2487	100.00	32	5.23
1	1.00, 2.01	151	6.07	12	2.57
2	2.01, 9.02	2253	90.59	32	5.20
3	9.02, 136	97	3.90	8	11.14

Positive Control

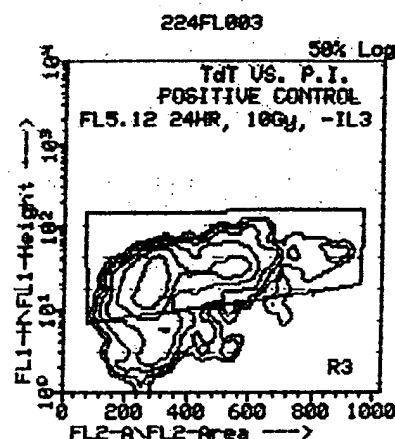
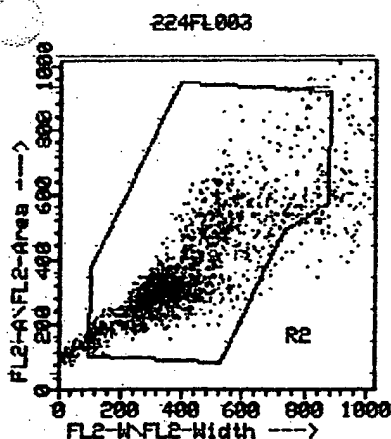
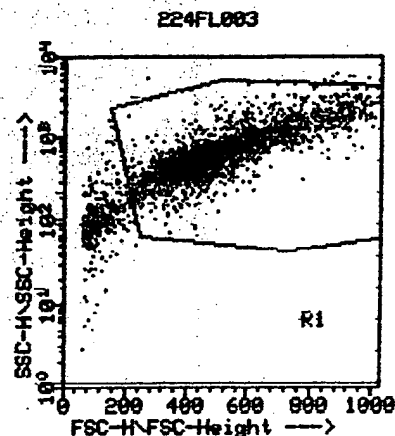
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:14:19

SELECTED PREFERENCES: Arithmetic/Linear

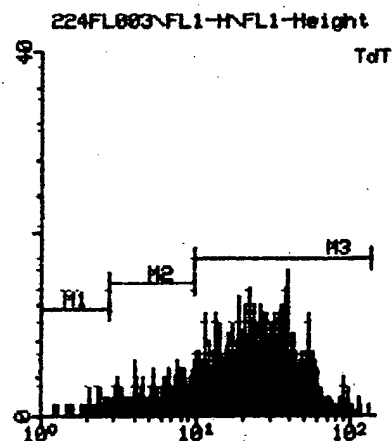


224FL003

Region Stats

File: 224FL003 Sample: FL5.12 CONTI
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1625

Rgn	Events	% Gated	% Total
1 R1	1625	100.00	54.17
2 R2	1625	100.00	54.17
3 R3	1335	82.15	44.50



224FL003-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 224FL003

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

H	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1625	100.00	16	21.87
1	1.00, 2.01	49	3.02	3	2.37
2	2.01, 9.02	274	16.86	6	6.13
3	9.02, 136	1307	80.43	16	26.42

THE UNIVERSITY OF CHICAGO
ANIMAL RESOURCES CENTER

REQUEST FOR TRANSFER OF ANIMAL OWNERSHIP

Requested transfer date _____

We request the following animals be transferred:

SPECIES nude mice QUANTITY 56

CAGE CARD NUMBER(S) _____

AA113928 through AA113943 (16 cages)

REQUESTED TRANSFER FROM:

PRINCIPAL INVESTIGATOR _____

PROTOCOL _____ FAS ACCOUNT _____

PHONE _____ SIGNATURE _____

Is this animal(s) been used in research or teaching? YES NO

If yes how? _____

The number of unused animals transferred will be restored to the number available for ordering under this ACUP.

REQUESTED TRANSFER TO:

PRINCIPAL INVESTIGATOR HALLAHAN / SIBLER

PROTOCOL _____ FAS ACCOUNT _____

PHONE 2-0294 SIGNATURE [Signature]

Will this animal(s) be moved to a new housing site / room? ☒ YES NO

If yes where? CARLSON J-013

The number of animals transferred will be deducted from the number available for ordering under this ACUP.

ARC APPROVALS:

CLINICAL VETERINARIAN APPROVAL _____ DATE _____

Animals which have been used or which are to be moved to a different housing site require the approval of a clinical veterinarian.

BUSINESS OFFICE APPROVAL _____ DATE _____

TRANSACTION DATE _____

GCS ENTRY BY _____

ACUP No.: 58671B

ACUP Amendment-Supplemental Form A

Only the primary investigator of an ACUP is permitted to make changes to that ACUP. Please note that certain changes in an ACUP may affect other aspects of that ACUP, and should be reflected in this amendment. ACUPs with amendments require Institutional Animal Care and Use Committee (IACUC) review and approval. The IACUC reserves the right to determine whether proposed changes are substantive or not, and to request further information or a new ACUP application, as appropriate. When submitting an amendment, the Principal Investigator is required to review all of the details of the original ACUP and to assure the IACUC that all unamended details remain identical to the original ACUP. Please note that the amendment must be typed.

Investigator: Dennis Hallahan, M.D.

Department: Radiation Oncology

ACUP Number: 58671B

Original approval date:

The following changes are herein proposed for this protocol:

☐ staff involved

Note: Include the number of years of work experience with the species used in this protocol, and confirm that each individual has reviewed the University of Chicago Manual on Laboratory Animals Interim Guide. If anyone listed has less than one year of relevant experience, please briefly describe how they will be or have been trained.

☐ housing ☒ procedure ☐ agents used☐ number of animals ☐ change in co-investigator☐ site outside centrally managed animal facilities to which live animals are taken and route of animal transport☒ response to questions raised during the IACUC-ARC review process ☐ other

Provide sufficient details, including a rationale for the proposed change, in narrative form to allow the committee to make a decision. Use additional pages as necessary and submit revised pages of the original ACUP, as appropriate.

Clarification of endpoints used in protocol #58671B:

1.) Tumor Size: The cutoff as mentioned in the protocol is "10% of body weight". Tumor measurements are typically performed twice weekly and tumor volumes are estimated by the formula $1/2(\text{length} \times \text{width} \times \text{height})$. This is derived from the equation for the volume of a sphere $= 4\pi r^3/3 = 4r^3/3 = d^3/2 = (l \times w \times h)/2$. Since most nude mice weigh 18-25 grams, a tumor size of $2000 \text{ mm}^3 = 2 \text{ cc} = 2 \text{ grams}$ is approximately 10% of body weight. This will be used as the cutoff for tumor size and can be easily verified by those monitoring the animals.

2.) Ulceration of tumors: When rapidly growing tumors outgrow their blood supply, they typically undergo necrosis in the central portion of the tumor. This creates a necrotic cavity which may heal completely over time if the tumor is cured. This situation is also seen in humans, most commonly in patients undergoing treatment for head and neck cancers. In our experience, this central necrosis is not tender in nude mice and has not predisposed the animals to infection, weight loss or loss of limb function. We continue to use these latter three conditions as criteria to sacrifice the animals.



Investigator

Date

This form must be submitted bearing the original signature of the investigator.

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT

QUEST

#3155)

REQ NO 24129

ARC USE ONLY

P.O. #

ORDER DATE

REF #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: SHIPMENTS ON A BASISREQUEST BY: Greg Sibley, MD DATE: 2-02-94REQUESTORS PHONE NUMBER: 2-0294AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 2-73731-5100VENDOR: FCRIREQUESTED DELIVERY DATE: 2-02-94P.I.: HallenPROTOCOL: 5861PHONE: 2-0294SPECIES: MouseQUANTITY: 80STRAIN: Albino NudeSEX: M ☒ F EITHERWEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(FOODENTS ONLY)

SPECIAL REQUIREMENTS:

Mice to go to Carlson Brodhead
Wife Room J-013

PROCUREMENT DESK: 2-9364

HOUSE AT: ☒ CARLSON ☐ WYLER ☒ CLSC ☐ FMI ☐ OTHERJ-013

Apoptosis Assay - U87 cells

Purpose - To see if apoptosis is seen in virally treated groups at earlier timepoints and to see if apoptosis is seen with TNF+RT at other time points

Methods - T-150 flasks of U87 cells were grown to ~80% confluence

			7 AM	1 PM	5 PM	7 PM	5 PM	5 PM	5 PM	%A
			-10	-4	0°	6°	24°	48°	72°	
1	Control						Harvest			1.8
2	TNF+RT	6°		TNF	RT	Harvest				2.6
3	"	24°		TNF	RT	Harvest				10.1
4	"	48°		TNF	RT	Harvest				5
5	"	72°		TNF	RT			Harvest		16.
6	2x10⁷ 899-6	6°	infect			Harvest				4
10		24°	infect			Harvest				13.
7	2x10⁷ 899-6+RT	6°	infect		RT	Harvest				5.6
11		24°	infect		RT	Harvest				25
8	2x10⁷ 899-6	6°	infect			Harvest				13.7
12		24°	infect			Harvest				11.
9	2x10⁷ 899-6+RT	6°	infect		RT	Harvest				8.1
13		24°	infect		RT	Harvest				9

Infection - cells were infected in 6 ml of 199V media x 2° then additional 14 cc of 10 FCS media was added

RT - single fraction of 20 Cg

TNF - 10 ul of 0.01 mg/ml stock in 20 x.1 media

Control
⊖ TdT

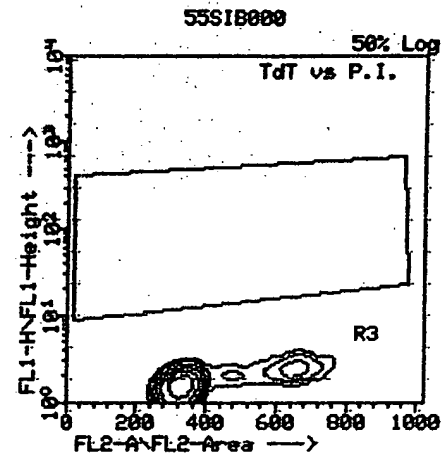
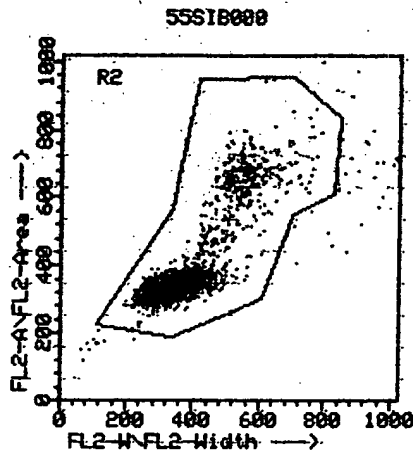
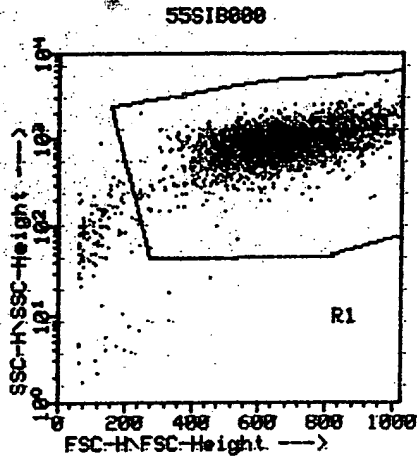
**BECTON
DICKINSON**

LYSYS II Ver 1.1

DATE:

TIME: 15:03:12

SELECTED PREFERENCES: Arithmetic/Linear



55SIB000

Region Stats

File: 55SIB000 Sample: SIBLEY APOP!

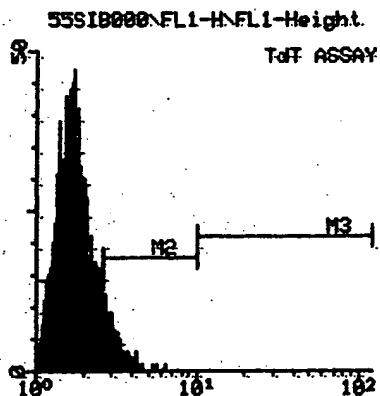
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Line

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total	
1 R1	2399	100.00	79.97	1.
2 R2	2399	100.00	79.97	
3 R3	0	0.00	0.00	



55SIB000\FL1-H\FL1-Height

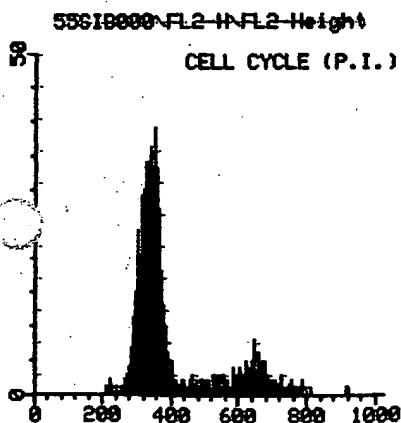
Arithmetic Histogram Statistics for 55SIB000

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M Left,Right Events % Peak Median

0	1.00, 9910	2399	100.00	47	1.70
1	1.00, 2.64	2202	91.79	47	1.67
2	2.64, 9.91	208	8.67	12	2.94
3	9.91, 118	0	0.00	0	—



0

Control
+ TdT

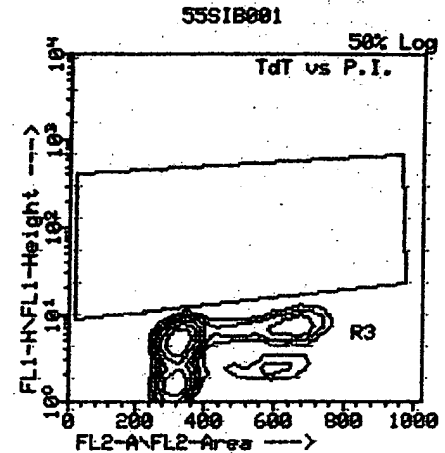
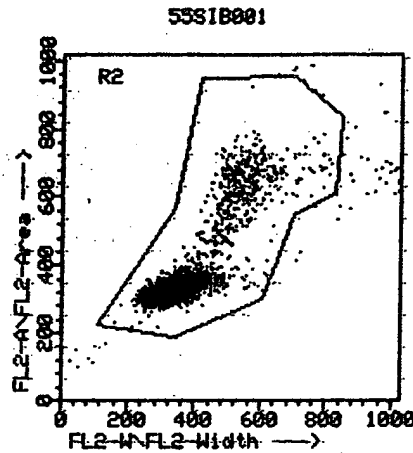
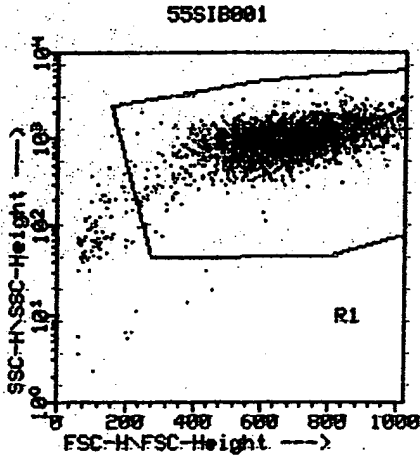
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:04:24

SELECTED PREFERENCES: Arithmetic/Linear



55SIB001

Region Stats

File: 55SIB001 Sample: SIBLEY APOP !

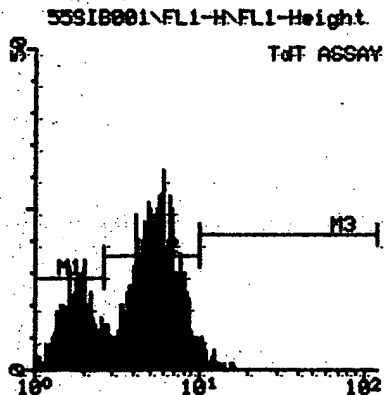
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total
1 R1	2399	100.00	79.97
2 R2	2399	100.00	79.97
3 R3	1	0.04	0.03



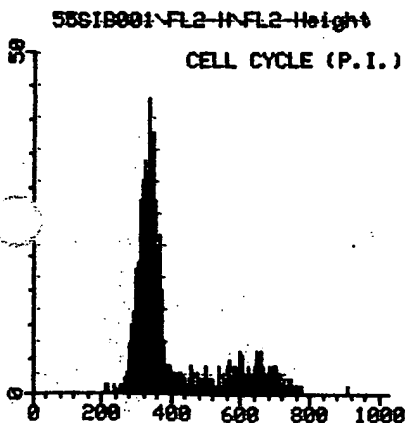
55SIB001\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

Left,Right	Events	%	Peak	Median
0 1.00, 9910	2399	100.00	31	4.74
1 1.00, 2.64	646	26.93	17	1.78
2 2.64, 9.91	1717	71.57	31	5.42
3 9.91, 118	44	1.83	5	18.89



1.8

TNF+RT 6°

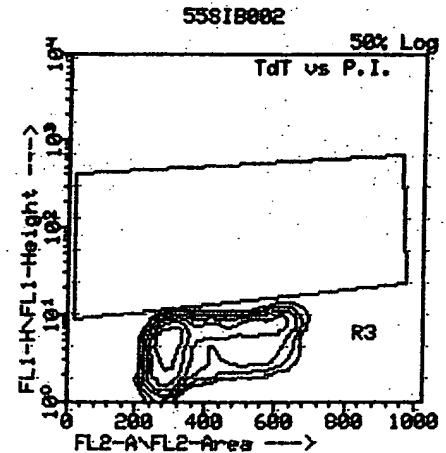
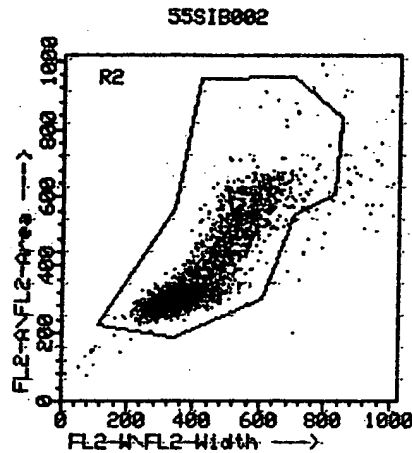
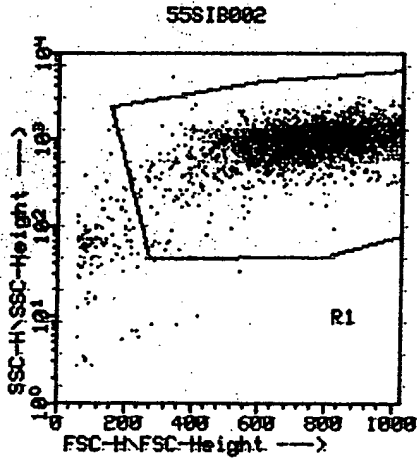
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:05:22

SELECTED PREFERENCES: Arithmetic/Linear



55SIB002

Region Stats

File: 55SIB002 Sample: SIBLEY APOP

Date: Gate G2= R1ANDR2

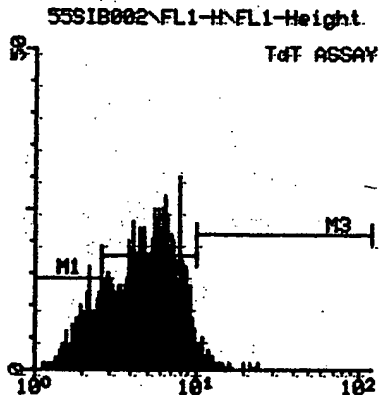
Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2450

Rgn Events % Gated % Total

Rgn	Events	% Gated	% Total
1 R1	2450	100.00	81.67
2 R2	2450	100.00	81.67
3 R3	4	0.16	0.13



55SIB002\FL1-H\FL1-Height

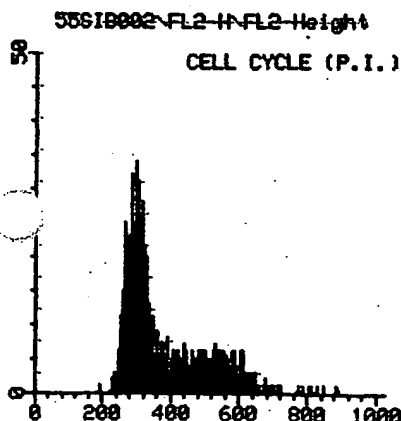
Arithmetic Histogram Statistics for 55SIB002

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M Left,Right Events % Peak Median

M	Left,Right	Events	%	Peak	Median
0	1.00, 99.10	2450	100.00	30	4.83
1	1.00, 2.64	400	16.33	16	2.15
2	2.64, 9.91	2003	81.76	30	5.28
3	9.91, 110	63	2.57	5	10.84



2.6

TdF+RT 24°

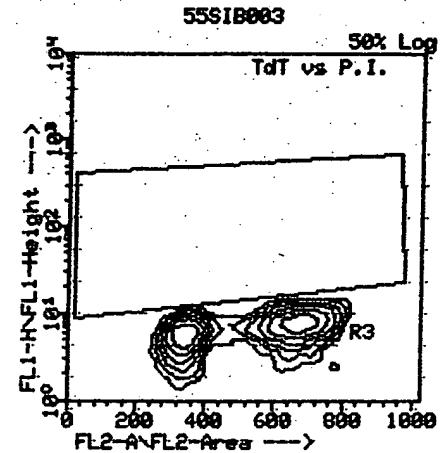
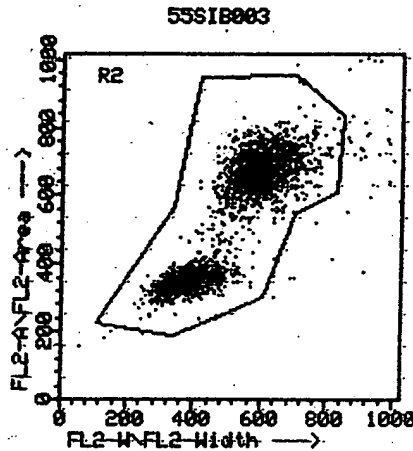
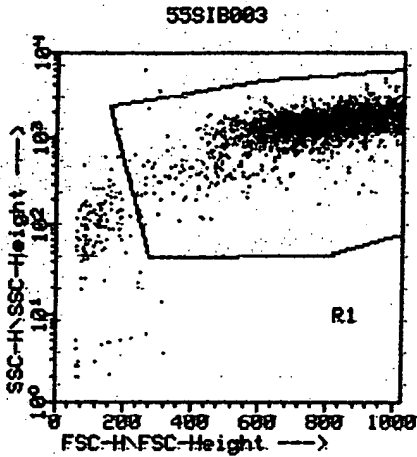
BECTON
DICKINSON

LYSYS II, Ver 1.1

DATE:

TIME: 15:06:21

SELECTED PREFERENCES: Arithmetic/Linear



55SIB003

Region Stats

File: 55SIB003 Sample: SIBLEY APOP

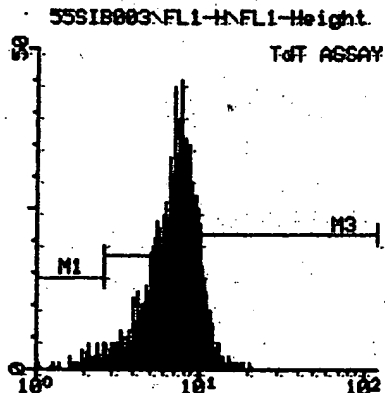
Date: Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2437

Rgn	Events	% Gated	% Total
1 R1	2437	100.00	81.23
2 R2	2437	100.00	81.23
3 R3	4	0.16	0.13



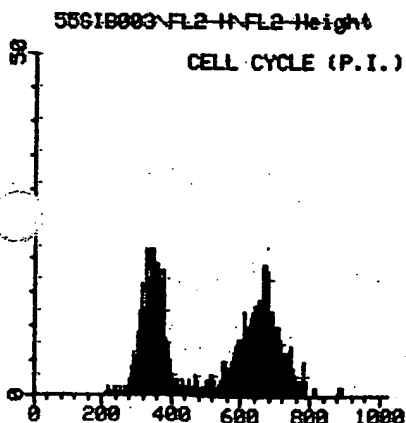
55SIB003\FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55SIB003

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2437	100.00	45	7.10
1	1.00, 2.64	56	2.30	4	2.19
2	2.64, 9.91	2148	88.14	45	6.92
3	9.91, 118	248	10.18	16	10.84



10.2

TNF+RT 480

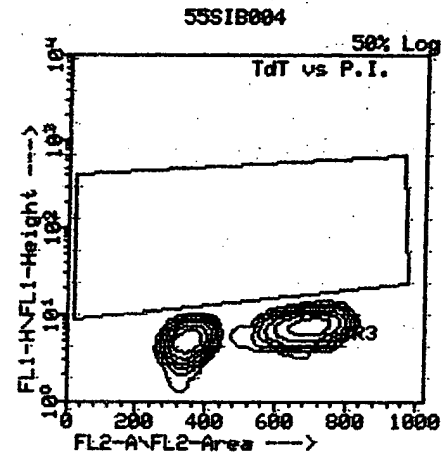
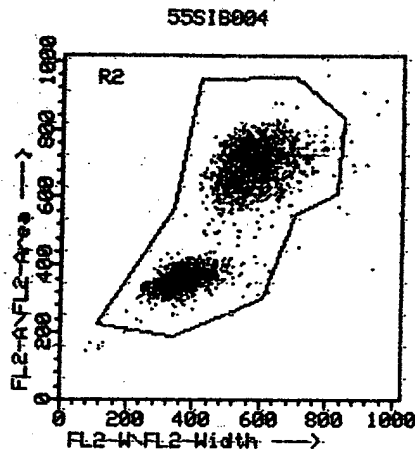
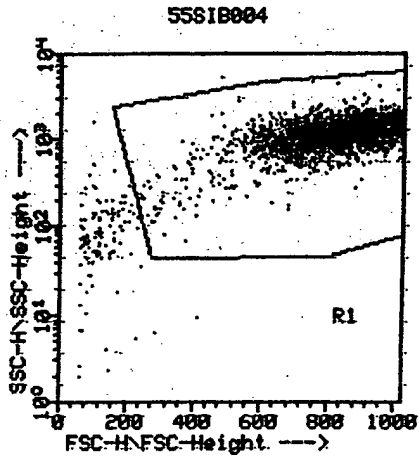
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

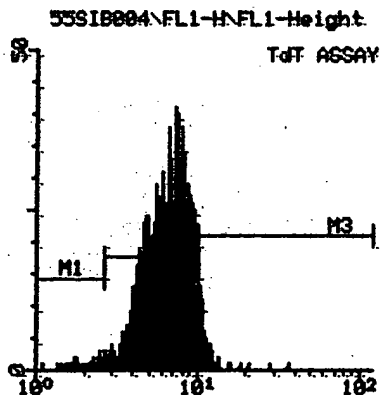
TIME: 15:07:20

SELECTED PREFERENCES: Arithmetic/Linear



55SIB004

Region Stats				
File: 55SIB004 Sample: SIBLEY APOP :				
Date: Gate G2= RIANDR2				
Selected Preferences: Arithmetic/Linear				
Parameters: FL2-A(LIN), FL1-H(LOG)				
Total= 3000 Gated= 2479				
Rgn	Events	% Gated	% Total	
1 R1	2479	100.00	82.63	2
2 R2	2479	100.00	82.63	1
3 R3	4	0.16	0.13	1



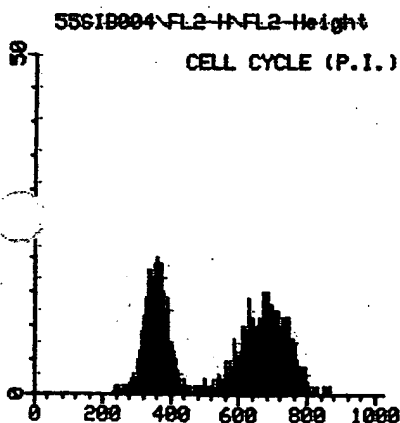
55SIB004\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB004

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2479	100.00	41	6.61
1	1.00, 2.64	35	41	3	2.09
2	2.64, 9.91	23	93	41	6.55
3	9.91, 118	1	04	13	10.46



5

TNF-RT 72°

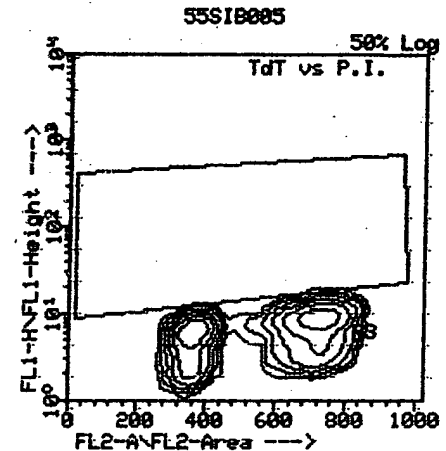
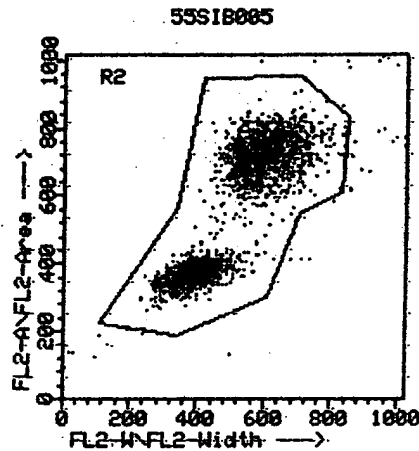
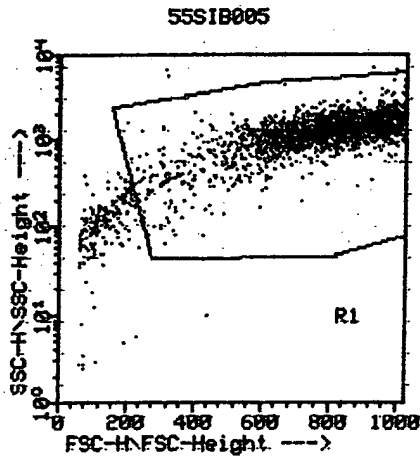
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:08:20

SELECTED PREFERENCES: Arithmetic/Linear



55SIB005

Region Stats

File: 55SIB005 Sample: SIBLEY APOP !

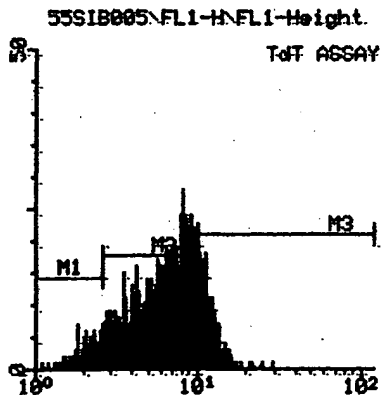
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2162

Rgn	Events	% Gated	% Total
1 R1	2162	100.00	72.07
2 R2	2162	100.00	72.07
3 R3	7	0.32	0.23



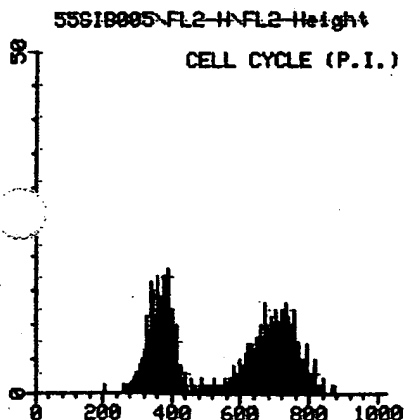
55SIB005-FL1-H-FL1-Height.

Arithmetic Histogram Statistics for 55SIB005

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2162	100.00	28	6.73
1	1.00, 2.64	167	7.72	7	2.19
2	2.64, 9.91	1656	76.60	28	6.38
3	9.91, 110	360	16.65	18	11.14



16.7

2x10⁶ 899-6: 6°

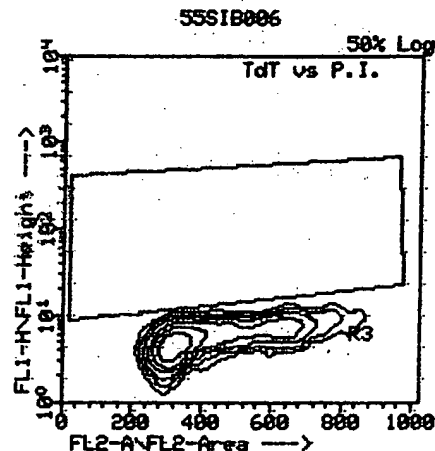
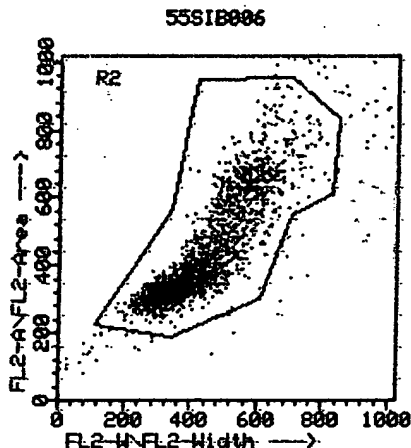
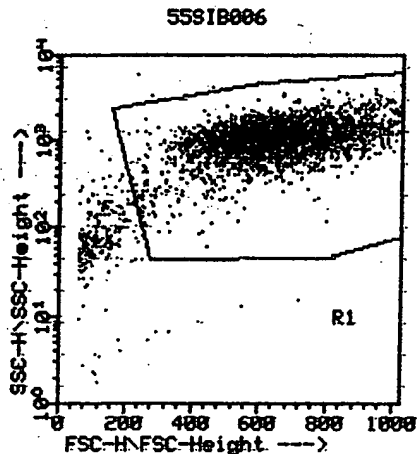
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:09:18

SELECTED PREFERENCES: Arithmetic/Linear

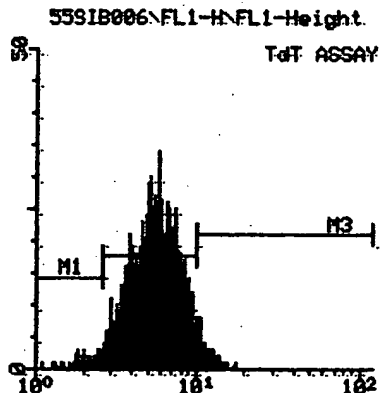


55SIB006

Region Stats

File: 55SIB006 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 2146

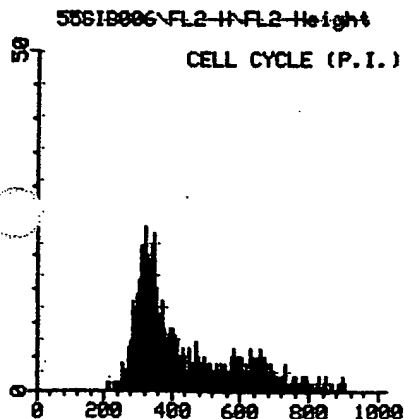
Rgn	Events	% Gated	% Total
1 R1	2146	100.00	71.53
2 R2	2146	100.00	71.53
3 R3	2	0.09	0.07



55SIB006\FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55SIB006
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2146	100.00	34	5.57
1	1.00, 2.64	56	2.61	3	2.11
2	2.64, 9.91	2006	93.48	34	5.52
3	9.91, 118	93	4.33	8	10.65



2x10⁶ 879-6 + RT 6°

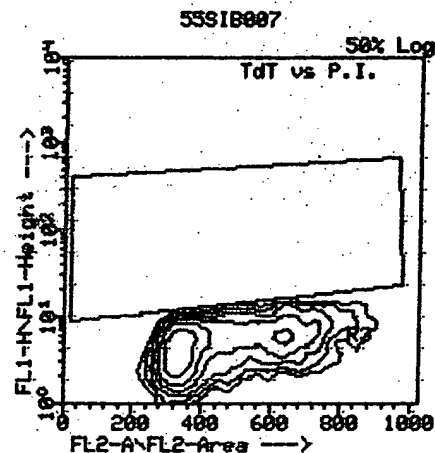
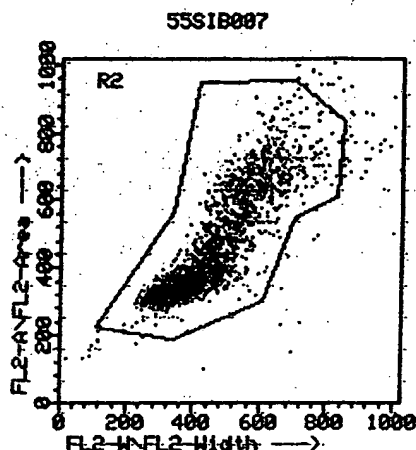
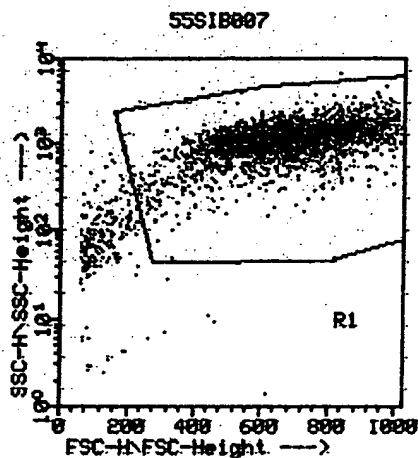
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:10:15

SELECTED PREFERENCES: Arithmetic/Linear

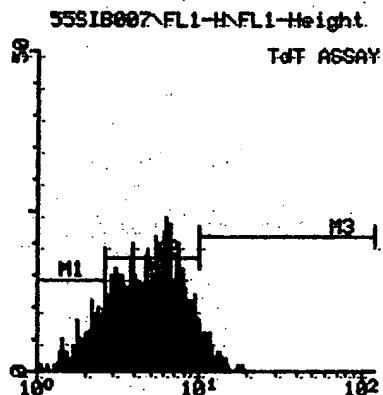


55SIB007

Region Stats

File: 55SIB007 Sampler: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 2128

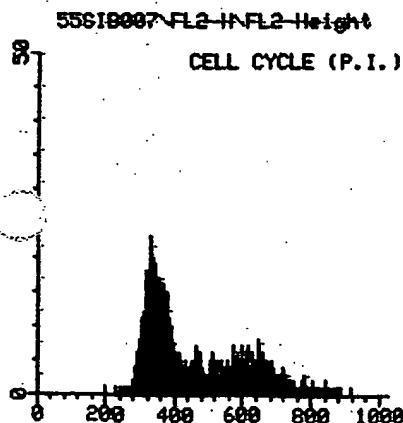
Rgn	Events	% Gated	% Total
1 R1	2128	100.00	70.93
2 R2	2128	100.00	70.93
3 R3	2	0.09	0.07



55SIB007/FL1-H/FL1-Height

Arithmetic Histogram Statistics for 55SIB007
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2128	100.00	24	4.87
1	1.00, 2.64	292	13.72	14	2.17
2	2.64, 9.91	1727	81.16	24	5.23
3	9.91, 118	120	5.64	8	11.24



5.6

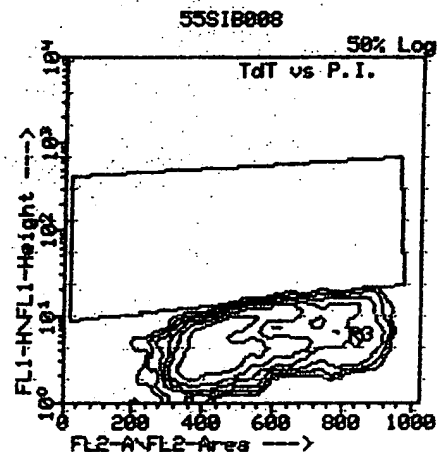
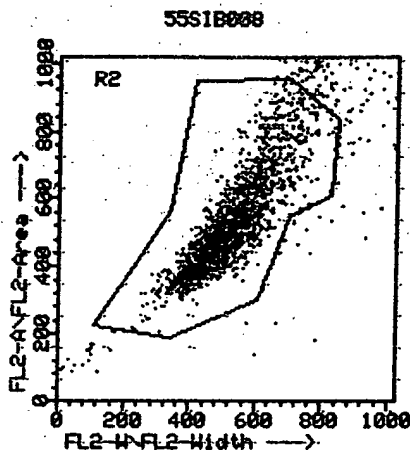
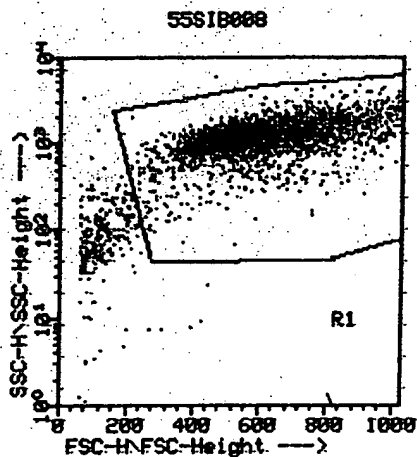
BECTION
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:11:13

SELECTED PREFERENCES: Arithmetic/Linear

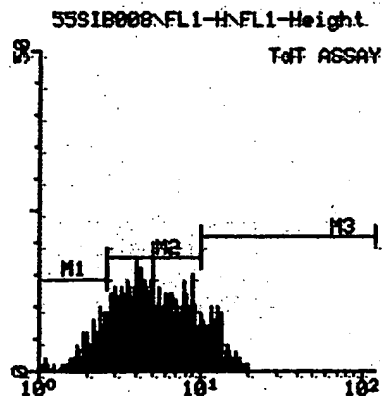


55SIB008

Region Stats

File: 55SIB008 Sampler SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1748
Rgn Events % Gated % Total :

1	R1	1748	100.00	58.27	1
2	R2	1748	100.00	58.27	1
3	R3	2	0.11	0.07	1



55SIB008 FL1-H-FL1-Height

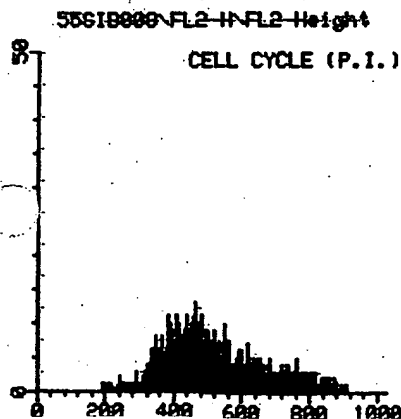
Arithmetic Histogram Statistics for 55SIB008

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M Left,Right Events % Peak Median

	M Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	20	4.78
1	1.00, 2.64	225	12.87	10	2.23
2	2.64, 9.91	1298	74.26	20	4.74
3	9.91, 118	240	13.73	10	11.92



13.7

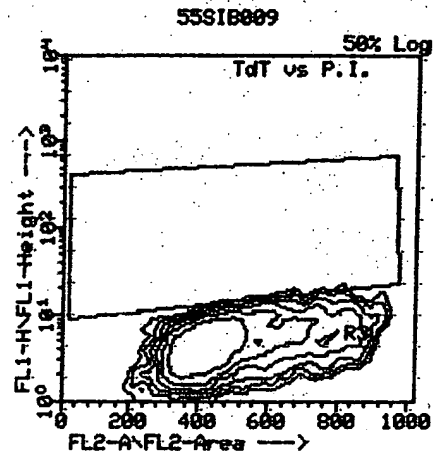
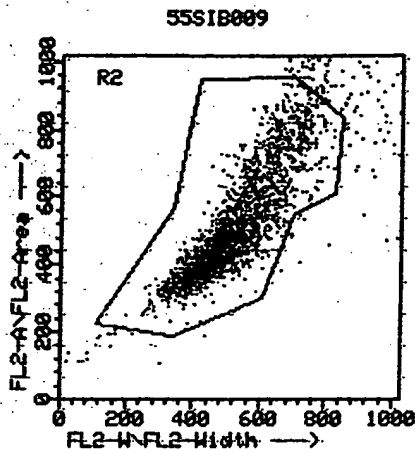
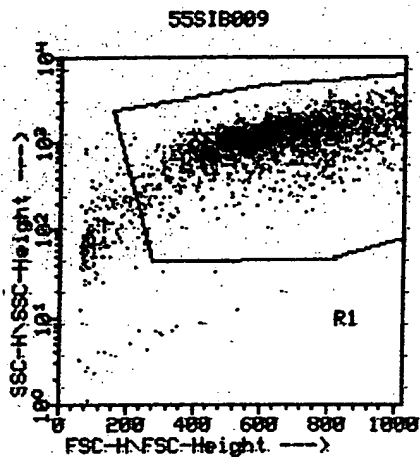
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:12:14

SELECTED PREFERENCES: Arithmetic/Linear

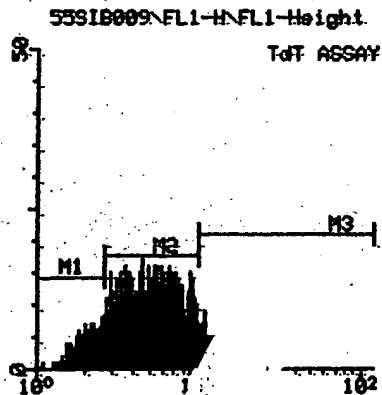


55SIB009

Region Stats

File: 55SIB009 Sample: SIBLEY APOP !
Date: Gate G2= RIANDR2
Selected Preferences: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1817
Rgn Events % Gated % Total

1 R1	1817	100.00	60.57	1
2 R2	1817	100.00	60.57	1
3 R3	6	0.33	0.20	1



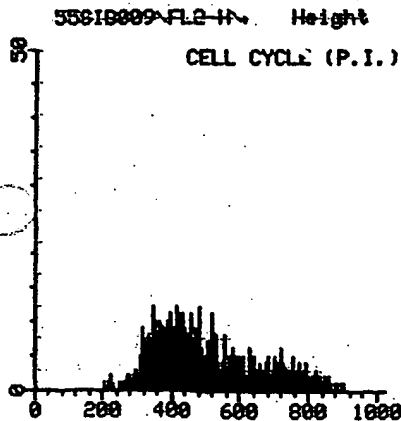
55SIB009\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB009

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1817	100.00	17	5.00
1	1.00, 2.64	236	12.99	8	2.13
2	2.64, 9.91	1435	78.98	17	5.19
3	9.91, 118	156	8.59	9	11.44



8.6

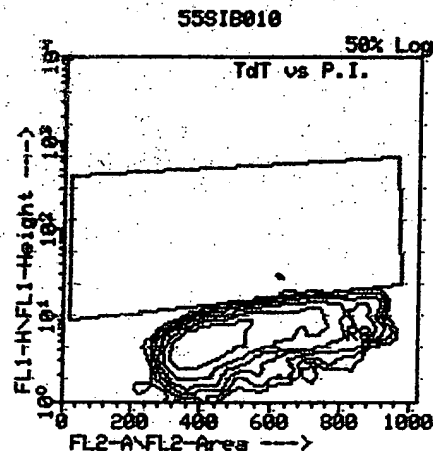
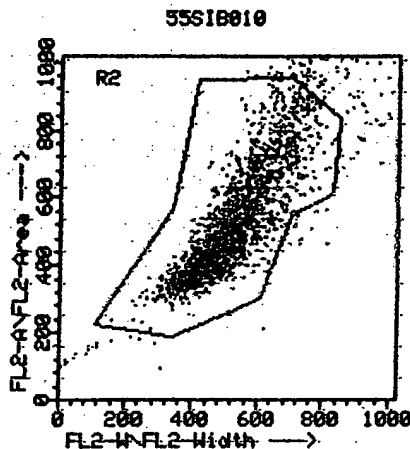
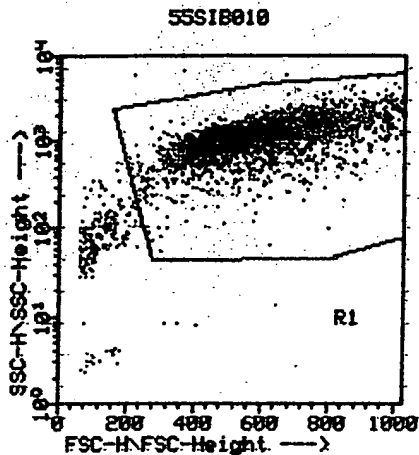
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:13:13

SELECTED PREFERENCES: Arithmetic/Linear



55SIB010

Region Stats

File: 55SIB010 Sample: SIBLEY APOP !

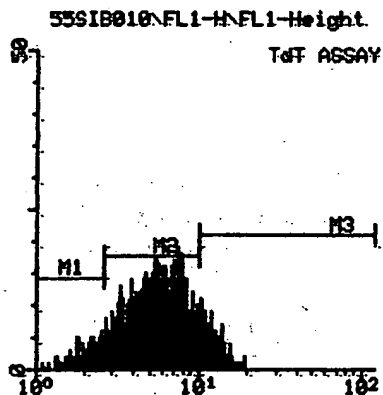
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 1748

Rgn	Events	% Gated	% Total
1 R1	1748	100.00	58.27
2 R2	1748	100.00	58.27
3 R3	1	0.06	0.03



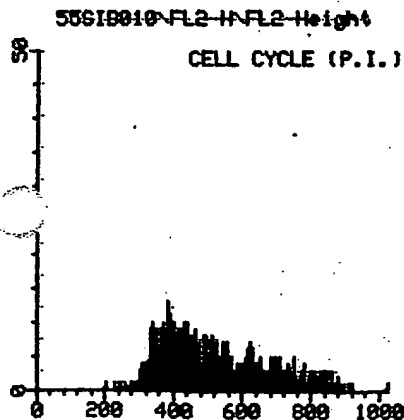
55SIB010-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55SIB010

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	19	5.62
1	1.00, 2.64	157	8.98	6	2.15
2	2.64, 9.91	1369	78.32	19	5.52
3	9.91, 110	235	13.44	11	11.76



13.4

2008-09-06 + RT 24°

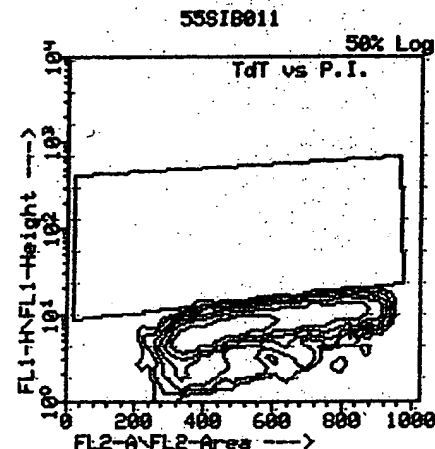
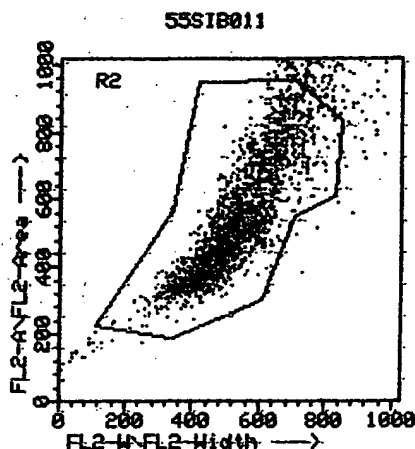
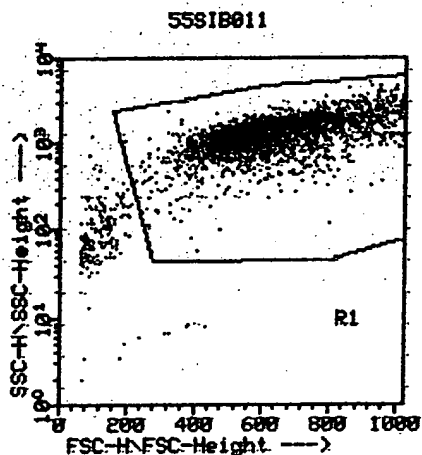
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:14:11

SELECTED PREFERENCES: Arithmetic/Linear



55SIB011

Region Stats

File: 55SIB011 Sample: SIBLY APOP !

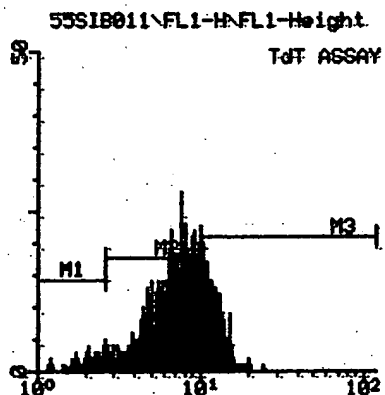
Date: Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 1815

Rgn	Events	% Gated	% Total
1 R1	1815	100.00	60.50
2 R2	1815	100.00	60.50
3 R3	5	0.28	0.17



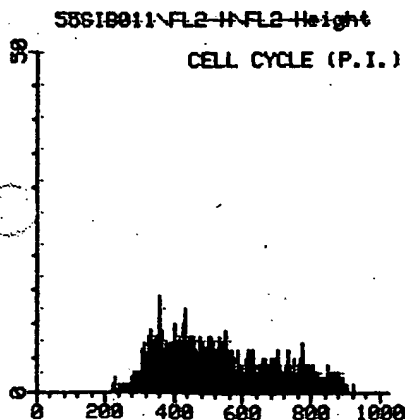
55SIB011\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB011

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1815	100.00	28	7.64
1	1.00, 2.64	95	5.23	5	2.09
2	2.64, 9.91	1290	71.07	28	6.92
3	9.91, 118	454	25.01	28	11.55



25

2x10⁷ 898-6 24^o

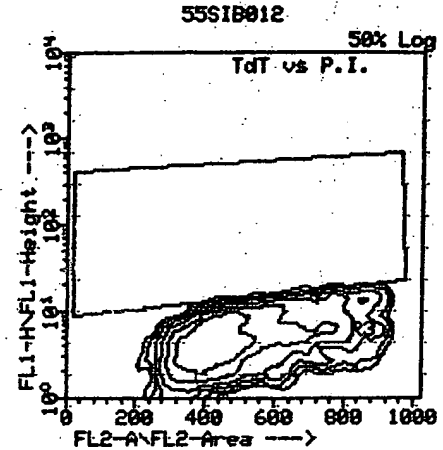
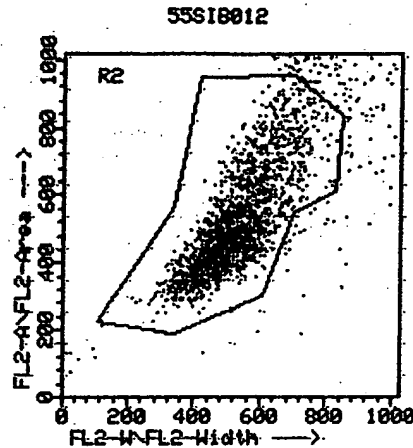
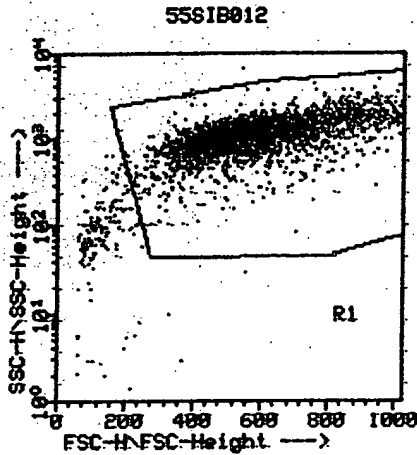
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:15:11

SELECTED PREFERENCES: Arithmetic/Linear



55SIB012

Region Stats

File: 55SIB012 Sample: SIBLEY APOP !

Date: Gate G2= R1ANDR2

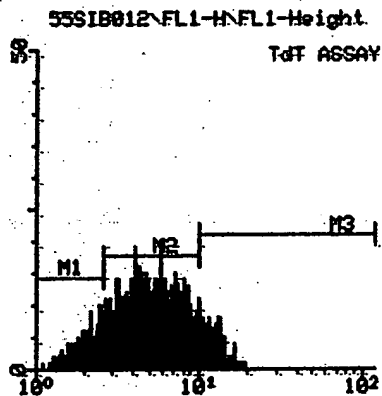
Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 1795

Rgn Events % Gated % Total

1 R1	1795	100.00	59.83	1:
2 R2	1795	100.00	59.83	1:
3 R3	3	0.17	0.10	1:



55SIB012\FL1-H\FL1-Height

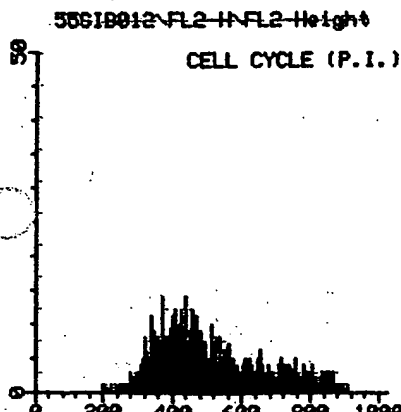
Arithmetic Histogram Statistics for 55SIB012

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M Left,Right Events % Peak Median

	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1795	100.00	19	4.91
1	1.00, 2.64	269	14.99	10	2.11
2	2.64, 9.91	1339	74.60	19	5.09
3	9.91, 110	205	11.42	11	11.65



11.4

2x10⁷ 89-6+RT 24

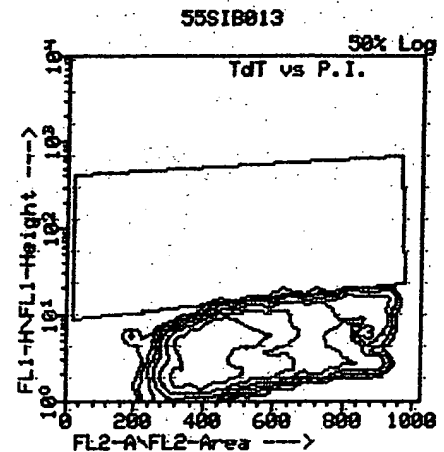
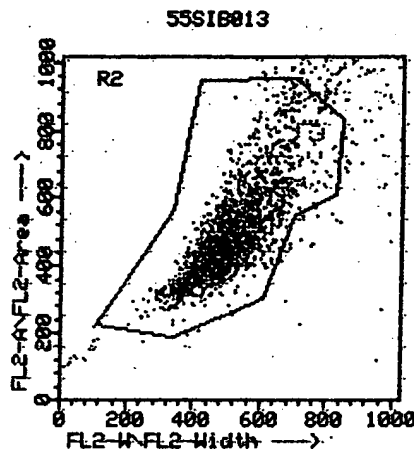
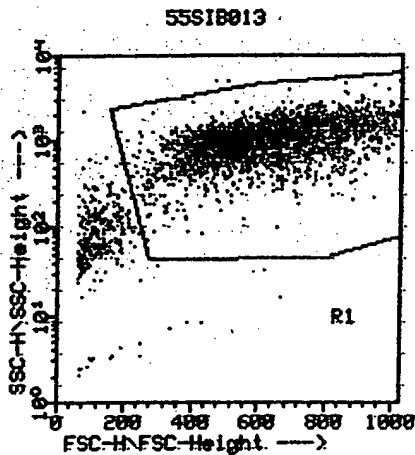
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:16:10

SELECTED PREFERENCES: Arithmetic/Linear

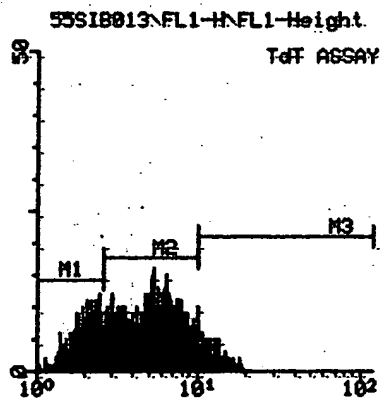


55SIB013

Region Stats

File: 55SIB013 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1622

Rgn	Events	% Gated	% Total
1 R1	1622	100.00	54.07
2 R2	1622	100.00	54.07
3 R3	4	0.25	0.13



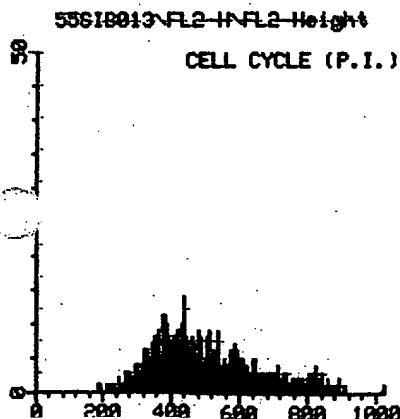
55SIB013\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB013

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1622	100.00	16	4.49
1	1.00, 2.64	416	25.65	12	2.07
2	2.64, 9.91	1074	66.21	16	5.19
3	9.91, 118	146	9.00	10	11.86



9

Pos Control
 @ TdT

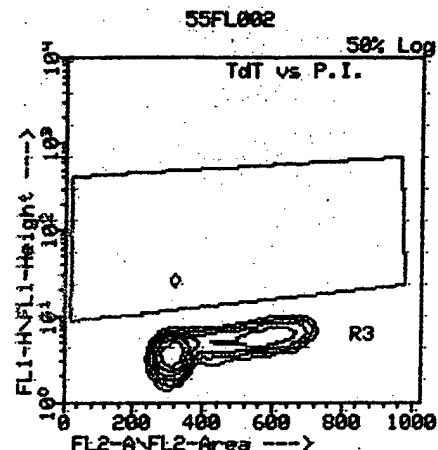
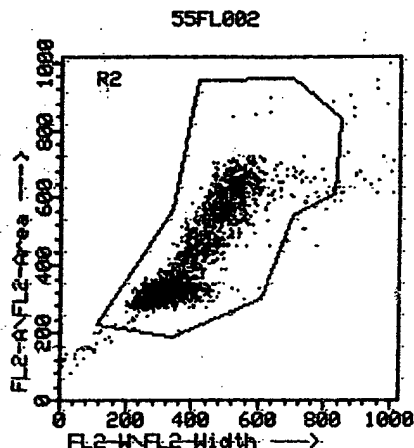
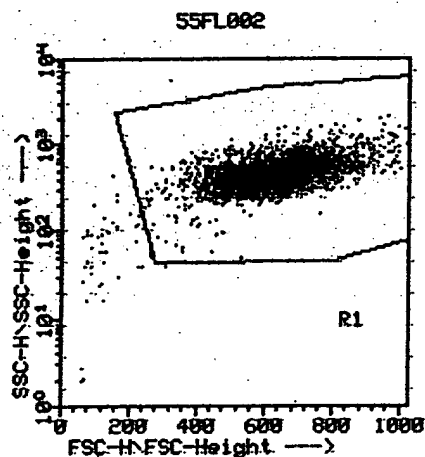
BECTON
 DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:19:50

SELECTED PREFERENCES: Arithmetic/Linear

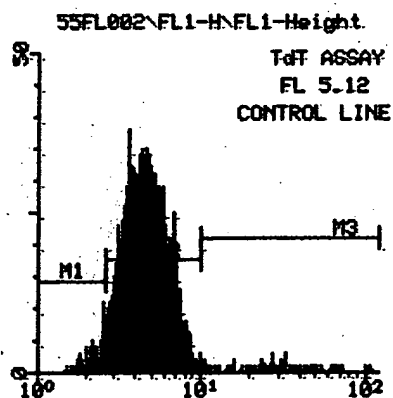


55FL002

----- Region Stats -----

File: 55FL002 Sample: SIBLEY APOP 5
 Date: Gate G2= R1ANDR2
 Selected Preference: Arithmetic/Line
 Parameters: FL2-A(LIN), FL1-H(LOG)
 Total= 3000 Gated= 2684

Rgn	Events	% Gated	% Total
1 R1	2684	100.00	89.47
2 R2	2684	100.00	89.47
3 R3	58	2.16	1.93



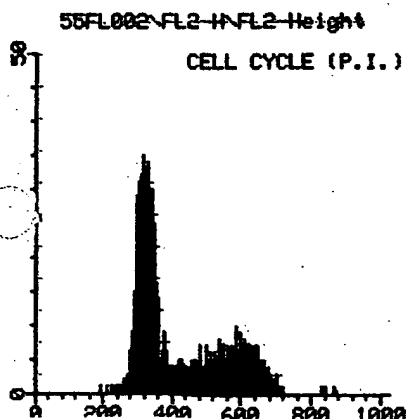
55FL002\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55FL002 ---

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2684	100.00	38	4.49
1	1.00, 2.64	121	4.51	11	2.35
2	2.64, 9.91	2501	93.18	38	4.53
3	9.91, 118	68	2.53	3	27.14



2.5

positive control
⊕ TdT

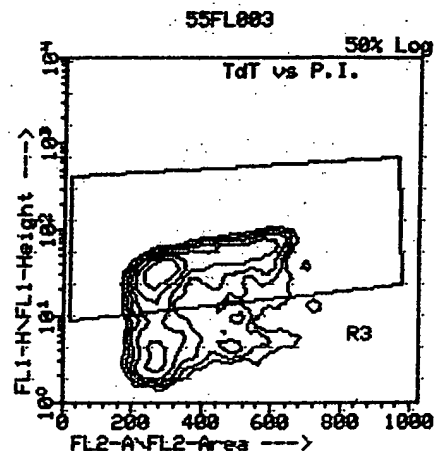
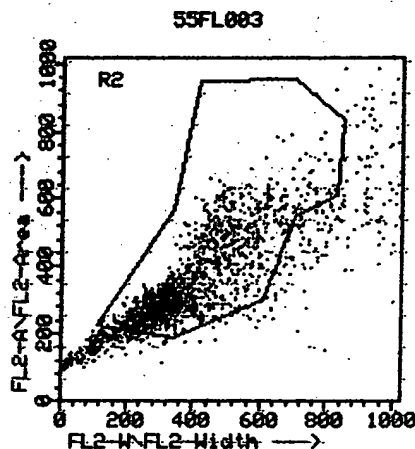
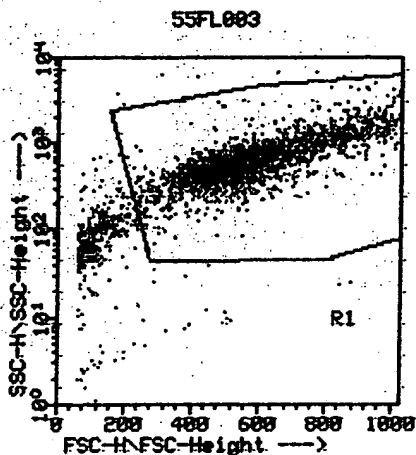
BECTON
DICKINSON

LYSYS II Ver 1.1

DATE:

TIME: 15:21:40

SELECTED PREFERENCES: Arithmetic/Linear

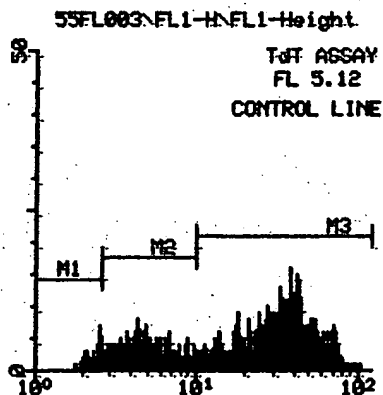


55FL003

Region Stats

File: 55FL003 Sample: SIBLEY APOP 5.
Date: Date G2= R1ANDR2
Selected Preferences: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1439

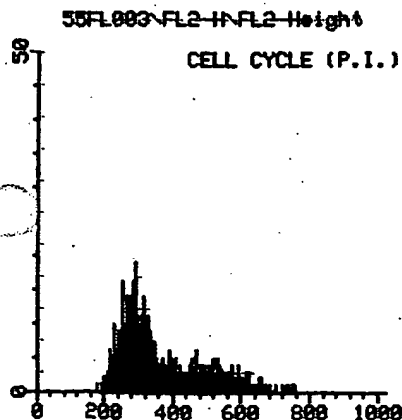
Rgn	Events	% Gated	% Total
1 R1	1439	100.00	47.97
2 R2	1439	100.00	47.97
3 R3	962	66.85	32.07



55FL003\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55FL003 -
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1439	100.00	16	25.71
1	1.00, 2.64	56	3.89	7	2.44
2	2.64, 9.91	374	25.99	8	4.96
3	9.91, 118	1012	70.33	16	34.29



70.3

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	0	0	0					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	0	0	0					
2057	AA111332	10^7 899-6								
1709	AA104549	10^7 3616								
1723	AA104549	10^7 3616	0	0	0					
2041	AA111334	10^7 3616								
2064	AA111334	10^7 3616								
1859	AA108972	RT Alone	12.5	11.5	9					
1861	AA108972	RT Alone								
1862	AA108972	RT Alone	6	5	4					
1892	AA108964	RT Alone	6	4.5	3					
1863	AA108971	10^7 3616+RT	-4.5	4	3.5					
1864	AA108971	10^7 3616+RT	-17	9.5	7					
1865	AA108971	10^7 3616+RT	-4.5	3.5	1					
1866	AA108971	10^7 3616+RT	-4	5	1					
1883	AA108966	10^7 3616+RT	-0	0	0					
1884	AA108966	10^7 3616+RT	-1	1	1					
1886	AA108966	10^7 3616+RT	-0	0	0					
2074	AA108966	10^7 3616+RT	-9.5	8.5	6					
2032	AA111326	10^7 3616+RT	-6	5	4.5					
2033	AA111326	10^7 3616+RT	-5	4	3					
2035	AA111326	10^7 3616+RT	-8	7	4.5					
2072	AA111326	10^7 3616+RT	0	0	0					
2036	AA111327	10^7 3616+RT	-7	6	3.5					
2037	AA111327	10^7 3616+RT	-4	3	1.5					
2039	AA111327	10^7 3616+RT	9	8	5					
2073	AA111327	10^7 3616+RT	-4	5	1.5					
1871	AA108969	10^7 899-6+RT	3.5	3.5	2					
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	-5	7	4.5					
1874	AA108969	10^7 899-6+RT	-0	0	0					
1880	AA108967	10^7 899-6+RT	-0	0	0					
1881	AA108967	10^7 899-6+RT	0	0	0					
18	AA108967	10^7 899-6+RT	2.5	2	1					
20	AA111329	10^7 899-6+RT	4.5	3.5	2.5					
2046	AA111329	10^7 899-6+RT	0	0	0					
2047	AA111329	10^7 899-6+RT	4.5	5	2.5					
2048	AA111330	10^7 899-6+RT	-2.5	2.5	1.5					
2049	AA111330	10^7 899-6+RT	16	13.5	11.5					
2050	AA111330	10^7 899-6+RT	2.5	3.5	2					
2051	AA111330	10^7 899-6+RT	9	8	4.5					

[illegible]

LOG SHEETS

1710	AA105283	10^7 899-6	<10 d35	0	23.7		
2075	AA105283	10^7 899-6	<10 d42	0	23.0		
1713	AA105283	10^7 899-6	<10 d14	0	22.2		
2054	AA111331	10^7 899-6	<10 d49	0	23.4		
1709	AA104549	10^7 3616	<10 d31				
1723				0	24.3		
1859	AA108972	RT Alone		12.5x11x10	24.0		
1862	AA108972	RT Alone		5-5.3	25.1		
1892	AA108964	RT Alone		5x4x2	24.4		
2189	AA113941	RT Alone		10x11.5x8	22.0		
2190	AA113941	RT Alone		11.5x9.5x7	18.2		
2191	AA113941	RT Alone		12.5x8x6.5	18.6		
2192	AA113941	RT Alone		12x10x8	22.0		
2201	AA113944	RT Alone		8.5x10x8	22.6		
2202	AA113944	RT Alone		8.5x10x7	21.3		
2203	AA113944	RT Alone		8x7x6	24.3		
2204	AA113944	RT Alone		12.5x12.5x10	23.1		
1863	AA108971	10^7 3616+RT		5.5x4x3	24.7		
1864	AA108971	10^7 3616+RT		13.5x10x6.5	27.6		
1865	AA108971	10^7 3616+RT	<10 d38	4x3x1	27.0		
1866	AA108971	10^7 3616+RT	<10 d31	0	26.3		
1883	AA108966	10^7 3616+RT	<10 d24	0	22.6		
1884	AA108966	10^7 3616+RT	<10 d56	2x2x1.5	23.9		
1886	AA108966	10^7 3616+RT	<10 d31	0	21.5		
2074	AA108966	10^7 3616+RT		9.5x9x6	17.0		
2032	AA111326	10^7 3616+RT		6x7.5x4.5	22.1		
2033	AA111326	10^7 3616+RT		4x4x2.5	25.0		
2035	AA111326	10^7 3616+RT		7x8.5x5	22.2		
2072	AA111326	10^7 3616+RT	<10 d21	0	24.7		
2036	AA111327	10^7 3616+RT		6x7.5x4.5	22.1		
2037	AA111327	10^7 3616+RT	<10 d52	3x2x1	25.3		
2039	AA111327	10^7 3616+RT		8.5x9x5.5	17.6		
2073	AA111327	10^7 3616+RT		4x4x1	20.6		
				6.5x6x3	22.8		
1871	AA108969	10^7 899-6+RT	<10 d66	3.5x3.5x2	24.0		
1873	AA108969	10^7 899-6+RT		6x6x4	24.6		
1874	AA108969	10^7 899-6+RT	<10 d52	0	27.8		
1880	AA108967	10^7 899-6+RT	<10 d14	0	21.7		
1881	AA108967	10^7 899-6+RT	<10 d42	0	26.4		
1882	AA108967	10^7 899-6+RT	<10 d24	0	24.0		
2044	AA111329	10^7 899-6+RT		4.5x4x3	23.8		
2045	AA111329	10^7 899-6+RT	<10 d21	0	27.3		
2046	AA111329	10^7 899-6+RT		4.5x4x2	25.0		
2048	AA111330	10^7 899-6+RT	<10 d38	2x2.5x1	15.9		
2049	AA111330	10^7 899-6+RT		18.5x14x13.5	25.2		
2050	AA111330	10^7 899-6+RT	<10 d31	3x2x1.5	23.9		
2051	AA111330	10^7 899-6+RT		7x8x3.5	24.0		

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31				
1723				0			
1859	AA108972	RT Alone	11.5 x 10 x 10.5				
1862	AA108972	RT Alone	4.5 x 5 x 3.5				
1892	AA108964	RT Alone	4.5 x 3.5 x 3				
2189	AA113941	RT Alone	10.5 x 11 x 9				
2190	AA113941	RT Alone	11.5 x 8 x 8.5				
2191	AA113941	RT Alone	10.5 x 7 x 6.5				
2192	AA113941	RT Alone	11 x 12 x 9				
2201	AA113944	RT Alone	10 x 9.5 x 9				
2202	AA113944	RT Alone	7.5 x 7 x 6.5				
2203	AA113944	RT Alone	8.5 x 10 x 7				
2204	AA113944	RT Alone	11.5 x 11 x 9				
1863	AA108971	10^7 3616+RT		4 x 5 x 3			
1864	AA108971	10^7 3616+RT		15 x 12 x 8			
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	2 x 2 x 1.5			
1886	AA108966	10^7 3616+RT	<10 d31	3 x 4 x 2.5 0			
2074	AA108966	10^7 3616+RT		8 x 9 x 4			
2032	AA111326	10^7 3616+RT	5 x 7 x 4.5	5 x 7 x 4.5			
2033	AA111326	10^7 3616+RT		4 x 4 x 2.5			
2035	AA111326	10^7 3616+RT		8 x 6 x 5			
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		5.5 x 6.5 x 3			
2037	AA111327	10^7 3616+RT	<10 d52	3 x 2.5 x 1.5			
2039	AA111327	10^7 3616+RT		10 x 8 x 6			
2073	AA111327	10^7 3616+RT		4 x 3 x 1			
1871	AA108969	10^7 899-6+RT	<10 d66	3 x 4 x 2.5			
1873	AA108969	10^7 899-6+RT		6 x 5 x 3			
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		5 x 4 x 4			
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		4 x 3.5 x 2.5			
2048	AA111330	10^7 899-6+RT	<10 d38	11.5 x 1 x 1			
2049	AA111330	10^7 899-6+RT		18 x 13 x 11			
2050	AA111330	10^7 899-6+RT	<10 d31	3 x 2 x 1			
2051	AA111330	10^7 899-6+RT		6 x 8 x 4.5			

OVER

1710	AA105283	10^7 899-6	<10 d35	0	0	0	23.8
2075	AA105283	10^7 899-6	<10 d42	0	0	0	22
1713	AA105283	10^7 899-6	<10 d14	0	0	0	20.8
2054	AA111331	10^7 899-6	<10 d49	0	0	0	22.4
1709	AA104549	10^7 3616	<10 d31	0	0	0	25.3
23							
1859	AA108972	RT Alone		12	14	10	24.5
1862	AA108972	RT Alone		5.5	9	3	24.4
1892	AA108964	RT Alone		5	5	3	23.5
2189	AA113941	RT Alone		11	12	8	22.0
2190	AA113941	RT Alone		12	9.5	7	17.8
2191	AA113941	RT Alone		12	8.5	6	19.2
2192	AA113941	RT Alone		12	11	9	20.3
2201	AA113944	RT Alone		11	12	9	22.2
2202	AA113944	RT Alone		6.5	6.5	5.5	24.2
2203	AA113944	RT Alone		11.5	10	8.5	20.4
2204	AA113944	RT Alone		12	11.5	7	19.9
1863	AA108971	10^7 3616+RT		5	4	2	23.3
1864	AA108971	10^7 3616+RT		14	13	7	25.4
1865	AA108971	10^7 3616+RT	<10 d38	0	0	0	25.6
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	25.2
1883	AA108966	10^7 3616+RT	<10 d24	0	0	0	22.1
1884	AA108966	10^7 3616+RT	<10 d56	0	0	0	23.9
1886	AA108966	10^7 3616+RT	<10 d31	0	0	0	21.4
2074	AA108966	10^7 3616+RT		10	9.5	4	16.8
2032	AA111326	10^7 3616+RT		8	7	5.5	23.2
2033	AA111326	10^7 3616+RT		4.5	4	1.5	25.1
2035	AA111326	10^7 3616+RT		0	0	0	24.4
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	4	23.3
2036	AA111327	10^7 3616+RT		6	6.5	3	23.8
2037	AA111327	10^7 3616+RT	<10 d52	5	3.5	1.5	25.2
2039	AA111327	10^7 3616+RT		11	9	6	16.8
2073	AA111327	10^7 3616+RT		0	0	0	20.9
1871	AA108969	10^7 899-6+RT	<10 d66	7	6	2.5	23.9
1873	AA108969	10^7 899-6+RT		5	5	3	23.3
1874	AA108969	10^7 899-6+RT	<10 d52	0	0	0	27.9
1880	AA108967	10^7 899-6+RT	<10 d14	0	0	0	21.2
1881	AA108967	10^7 899-6+RT	<10 d42	0	0	0	25.1
1882	AA108967	10^7 899-6+RT	<10 d24	6	5	1.5	23.9
2044	AA111329	10^7 899-6+RT		7	6	4.5	24.3
2045	AA111329	10^7 899-6+RT	<10 d21	3.5	3.5	2	25.4
2046	AA111329	10^7 899-6+RT		0	0	0	25.4
2048	AA111330	10^7 899-6+RT	<10 d38	0	0	0	16.2
2049	AA111330	10^7 899-6+RT		21.5	15	14	25.3
2050	AA111330	10^7 899-6+RT	<10 d31	0	0	0	23.8
2051	AA111330	10^7 899-6+RT		8	10	5.5	24.6

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R 2090 9 8.5 7.5 22
LR

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
23							
1859	AA108972	RT Alone		12.5	19	10.5	
1862	AA108972	RT Alone		5	5	3.5	
1892	AA108964	RT Alone		4.5	5	2.5	
2189	AA113941	RT Alone		11	11.5	8	
2190	AA113941	RT Alone		9	12	6.5	
2191	AA113941	RT Alone		8	11	4	
2192	AA113941	RT Alone		11	11	7	
2201	AA113944	RT Alone		11	11	7	
2202	AA113944	RT Alone		7	7	5	
2203	AA113944	RT Alone		11.5	12	9	
2204	AA113944	RT Alone		12	12	8.5	
1863	AA108971	10^7 3616+RT		4	5	2	
1864	AA108971	10^7 3616+RT		14	11.5	6	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	12 0	12 0	6 0	
2074	AA108966	10^7 3616+RT		13	10	6	
2032	AA111326	10^7 3616+RT		6.5	8	6	
2033	AA111326	10^7 3616+RT		4	3.5	2	
2035	AA111326	10^7 3616+RT		0			
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	6	
2036	AA111327	10^7 3616+RT		6	7	2.5	
2037	AA111327	10^7 3616+RT	<10 d52	4	4	2	
2039	AA111327	10^7 3616+RT		12	10	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	6	5.5	2.5	
1873	AA108969	10^7 899-6+RT		5	5	3	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		6.5	6	4	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3.5	3.5	2	
2048	AA111330	10^7 899-6+RT	<10 d38	3.5	3	1	
2049	AA111330	10^7 899-6+RT		22000			
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		7.5	10	5	

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2207 11 11.5 9

OVER

died
drown

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			23.2
1713	AA105283	10^7 899-6	<10 d14	0			22.3
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1859	AA108972	RT Alone		14	13.5	10	25
1862	AA108972	RT Alone		5	5	4	24.4
1892	AA108964	RT Alone		5	4	3	23.8
2189	AA113941	RT Alone		10.5	10	8	20.9
2190	AA113941	RT Alone		11	8	6.5	18.2
2191	AA113941	RT Alone					
2192	AA113941	RT Alone		11	9	7	22.2
2201	AA113944	RT Alone		10.5	10.5	8	20.3
2202	AA113944	RT Alone		6.5	5	4	24
2203	AA113944	RT Alone		10	10	7	20.6
2204	AA113944	RT Alone		11	11.5	8.5	21.1
1863	AA108971	10^7 3616+RT		4	3	3	23.8
1864	AA108971	10^7 3616+RT		13.5	12	7	25.3
1865	AA108971	10^7 3616+RT	<10 d38	0			26.4
1866	AA108971	10^7 3616+RT	<10 d31	0			26.2
1883	AA108966	10^7 3616+RT	<10 d24	0			22.2
1884	AA108966	10^7 3616+RT	<10 d56	2	1	1	24.2
1886	AA108966	10^7 3616+RT	<10 d31	0			26.2
2074	AA108966	10^7 3616+RT		16	11	7	17
2032	AA111326	10^7 3616+RT		7.5	5.5	4.5	23.3
2033	AA111326	10^7 3616+RT		4	3	2.5	25.7
2035	AA111326	10^7 3616+RT		9.5	7	6	23.9
2072	AA111326	10^7 3616+RT	<10 d21	0			25.5
2036	AA111327	10^7 3616+RT		6	5	4	26.6
2037	AA111327	10^7 3616+RT	<10 d52	3	3	1.5	26
2039	AA111327	10^7 3616+RT		10	8.5	6	18
2073	AA111327	10^7 3616+RT		0			21.6
1871	AA108969	10^7 899-6+RT	<10 d66	5	4	2	25.4
1873	AA108969	10^7 899-6+RT		7	5	4	24.6
1874	AA108969	10^7 899-6+RT	<10 d52	0			28.3
1880	AA108967	10^7 899-6+RT	<10 d14	0			22.0
1881	AA108967	10^7 899-6+P	<10 d42	6	5	3.5	26.6
1882	AA108967	10^7 899-6+P	<10 d24	0			24.2
2044	AA111329	10^7 899-6+R.		5	6.5	4.5	25.8
2045	AA111329	10^7 899-6+RT	<10 d21	0			16.7
2046	AA111329	10^7 899-6+RT		3.5	3	2	24.9
2048	AA111330	10^7 899-6+RT	<10 d38	0			15.9
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	5	3	2	25.6
2051	AA111330	10^7 899-6+RT		8.5	10.5	7.5	24.7

IN VIVO TNF PRODUCTION

	1	2	3	4	5	6	7	8	9
	Group	Mouse	Time	Tum weight	Vol Buffer	[TNF]	ETNF/tumor	TNF/ mg-tumor	
1									
2									
3	2x10 ⁷ PRV 899-6	2370	3d	.45					
4	"	2371	3d	.26					
5	"	2372	3d	.37					
6	"	2373	3d	.44					
7									
8	2x10 ⁷ 899-6	2374	10d	.13					
9	"	2375	10d	.54					
10	"	2376	10d	.61					
11	"	2377	10d	.23					
12									
13	Control	2386	10d	2.41					
14	"	2387	10d	.35					
15	"	2388	10d	.47					
16	"	2389	10d						
17									
18	2x10 ⁷ 3616	2390	3d	0.44					
19	"	2391	3d	1.23					
20	"	2392	10d	.24					
21	"	2393	10d	.69					
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

INDUCTION- in vivo							
Mouse	Treatment	Tumor Wt	Vol Buffer	Reading	[TNF]pg/ml	[TNF]/tumor	[TNF]/n
2370	3d 899-6	0.45	1	0.679	331	480	1.0661
2371	3d 899-6	0.26	1	0.708	348	439	1.687
2372	3d 899-6	0.37	1	1.326	747	1023	2.7641
2373	3d 899-6	0.44	1	1.42	811	1168	2.6553
2374	10d 899-6	0.13	1	0.352	149	168	1.2938
2375	10d 899-6	0.54	1	0.444	197	304	0.5629
2376	10d 899-6	0.61	1	0.274	110	177	0.2897
2377	10d 899-6	0.23	1	0.323	134	165	0.717
2386	10d Control	2.41	1	2.021	1246	4249	1.7632
2387	10d Control	0.35	1	1.823	1099	1484	4.2401
2388	10d Control	0.47	1	2.498	1612	2370	5.0426
2390	3d 3616	0.44	1	0.37	158	228	0.5176
2391	3d 3616	1.23	1	0.515	236	527	0.4286
2392	10d 3616	0.24	1	0.374	160	199	0.8279
2393	10d 3616	0.69	1	0.756	377	637	0.9234

$$y = 529.72 \times X^{1.2158}$$

$$y = .005863 \times X^{813391}$$

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The number of transformed cells was determined by the number of colonies obtained on the selective medium. The results are the mean of three independent experiments. Error bars represent standard deviation.

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$\begin{matrix} 1 & 2 & 3 \\ 4 & 5 & 6 \\ 7 & 8 & 9 \end{matrix}$

[illegible]

2025 RELEASE UNDER E.O. 14176

000 002

[illegible][illegible]

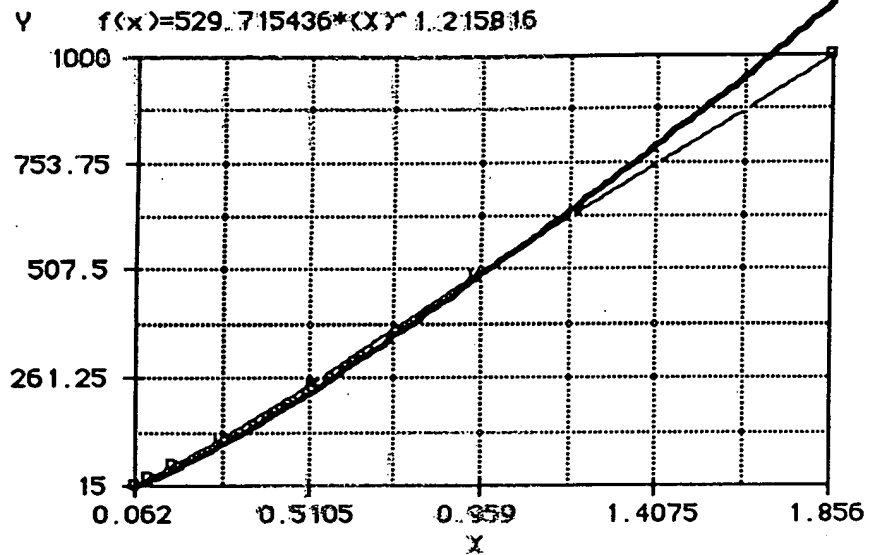
Modif./Inv. DATA:

.....X.....Y.....
0.062	15.13
0.095	31.13
0.158	62.5
0.282	125
0.518	250
0.943	500
1.856	1000

• EXPONENTIAL FIT •

$$Y = 529.715436 * (X)^{1.215816}$$

Coef. of Regression= 0.995013 For 7 Data Pairs

INITIALS Y.K.
DATE

SUBJECT • Untitled : [I: X<>Y]



CPU Time: 13 Secs.



Sikey

IMMUNOSTAINING & HISTOLOGY - U87

	1	2	3	4	5	6	7	8	9	
1										
2	Group		Mouse	Time	Prep					
3	2×10^7 PFV 899-6		2362	3d	formalin & RNA					
4	"		2363	3d	formalin & RNA					
5	"		2364	3d	frozen & RNA					
6	"		2365	3d	frozen & RNA					
7										
8	2×10^7 PFV 3616		2382	3d	formalin & RNA					
9	"		2383	3d	frozen & RNA					
10	"		2384	10d						
11	"		2385	10d						
12										
13	2×10^7 PFV 899-6		2366	10d						
14	"		2367	10d						
15	"		2368	10d						
16	"		2369	10d						
17										
18	Control		2378	3d	formalin & RNA					
19	"		2379	3d	frozen & RNA					
20	"		2380	10d	Em, formalin, frozen					
21	"		2381	10d						
22			2389	10d						
23										
24										
25										
26										
27										
28										
29										
30										
31										



1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1725							
1859	AA108972	RT Alone		13	13	11	
1862	AA108972	RT Alone		4.5	4	3	
1892	AA108964	RT Alone		4	4.5	2.5	
2189	AA113941	RT Alone		7	9.5	5.5	
2190	AA113941	RT Alone		9.5	10.5	7	
2191	AA113941	RT Alone					
2192	AA113941	RT Alone		9	10	6.5	
2201	AA113944	RT Alone		10	11	8.5	
2202	AA113944	RT Alone		5.5	4	2.5	
2203	AA113944	RT Alone		9	10	7.5	
2204	AA113944	RT Alone		11	11.5	7.5	
1863	AA108971	10^7 3616+RT		3.5	3	3	
1864	AA108971	10^7 3616+RT		13	12	7.5	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	0			
2074	AA108966	10^7 3616+RT		10	14	6	
2032	AA111326	10^7 3616+RT		6.5	7	5	
2033	AA111326	10^7 3616+RT		4	3	2	
2035	AA111326	10^7 3616+RT		7	8.5	5	
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		6	4.5	3.5	
2037	AA111327	10^7 3616+RT	<10 d52	0			
2039	AA111327	10^7 3616+RT		8	9	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	0			
1873	AA108969	10^7 899-6+RT		6	5	3.5	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	6	7.5	4	
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		8	6.5	5.5	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3	4.5	1.5	
2048	AA111330	10^7 899-6+RT	<10 d38	0			
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		13	9	9	

Control

2354	15.5	14.5	11.5	23.5
55	20	15.5	12	21.2
56	15.5	11	8.5	23.8
57	13	16	10	21.0

3616+RT

2032	8	6.5	7	23.9
33	3	3.5	2	25.8
2072	0			24.7
35	9	8	6	23.8

	1	2	3	4	5	6	7	8	9	
RT						3616+RT				
2342	14	14	6	20.9		2036	5.5	4.5	4	25.1
43	12.5	10.5	8	22.3		37	0			26.5
44	10	10	8	20.6		2073	0			21.3
45	12	10	7	21.2		39	8	7	4	21.2
RT						3616+RT				
2350	14	11.5	8	21.7		1883	0			21.6
51	9.5	7	6	22.7		84	0			23.9
52	10	9	7	23.6		2074	15.5	10	7	16.9
53	14	9	8	20.6		86	0			21.3
RT						3616+RT				
2189	9	9.5	7	20.6		1863	4	4	3.5	24.2
90	7	6	4	18.4		64	13	11	7	22.6
92	9.5	9	5.5	24.1		65	0			26.3
						66	0			25.3
RT						3616				
2201	10	10	9	19.3		1723	0			
02	2.5	2.5	1	26.6						
03	11.5	11	6	20.0						
04	12	11	7.5	23.7						
RT						899-6				
1859	12	13	11.5	26.3		2054	0			23.2
1892	4.5	3	2.5	24.3		2075	0			24.2
1862	5	4.5	4.5	25.1		1713	0			22
						1710	0			24.4
RT						899+RT				
2346	13.5	10.5	10	19.2		1880	0			21.4
47	12	10	7.5	23.0		81	8	5	4	27
48	16	10	9	18.6		82	0			24
49	9	11.5	7	22						
CONTROL						899+RT				
2206	17.5	20	16.5	24.9		2048	0			14.6
07	13	14.5	11.5	24.9		50	0			25.3
CONTROL						51	14	10	9	25.1
2358	16	15.5	14	20.9						
59	16	15.5	11.5	20.7						
60	17.5	12	11	23.4						
61	13	16	10	22						

RT Cont'd
 2354 17 17.5 11
 22 17.5 11.5
 56 16 14.5 8
 57 16.5 8.13 9

3616 + RT
 2032 8 8.5 6
 33 4 3 2.5
 2072 ~~22~~ 0
 35 9 7.5 6

EFFICIENCY LINE # 22-206



	1	2	3	4	5	6	7	8	9
1	RT 2342	15.5	13.5	9.5	3616 + RT				
2	2343	12	9	7	2032	5	4	3	
3	2344	13	11	7.5	2033	2.5	10.5	7	
4	2345	7.5	10	7.5	2034	0			
5					2035	0			
6	RT 2350	17	10	8.5	3616 + RT				
7	2351	11	8	7	2036	0			
8	2352	10	8	7	2037	2	1	1	
9	2353	14	9.5	7	? SAC → 2074	10	14	5.5	
10	RT 2189	10	10	7	2075	0			
11	2190	6	6	3.5	3616 + RT				
12	2192	9.5	10	5	2036	6	5	4	
13	RT 2201	9	11	8	2037	0			
14	2202	0			2073	0			
15	03	9.5	9.5	5	2039	8	7	4	
16	04	11	11.5	7					
17	RT 1859	13	12.5	11.5	899-6 + RT				
18	1860	11	6	3	? SAC → 2048	0			
19	1861	5	5	4	2050	6	4	1	
20					2051	12	15	9	
21	RT 2346	14	12	8.5	899 + RT				
22	2347	12	11	8	2052	0			
23	2348	15	10	9	2053	7	9.5	6	
24	2349	11.5	10	7	2054	0			
25	CONTROL				2055	0			
26	(2358)	19.5	18	14	2075	0			
27	59	18.5	17.5	12	2076	0			
28	60	15	18	12	2077	0			
29	61	15.5	17.5	12	2078	0			
30	CONTROL				899-6 + RT				
31	(2206)	20	22	18	2044	8	8.5	7	
	2207	14.5	14.5	13.5	45	ded			
					46	0			



The University of Chicago
Departmental Purchase Order

Purchase Order Number
Z904416
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

Phar Mosen
10975 Torreyana Rd
San Diego, CA 92121
City State Zip Code
Payment Terms Delivery charge? ☒ Yes ☐ No
Telephone No. *619-792-5233* FAX No. *619-792-5233*

Ship To

THE UNIVERSITY OF CHICAGO
Dept. Radiation Oncology
Greg Sibley, MD
5830 S. Ellis Ave
Chicago City State Zip Code
116 *60637*
Dept. Code: Room

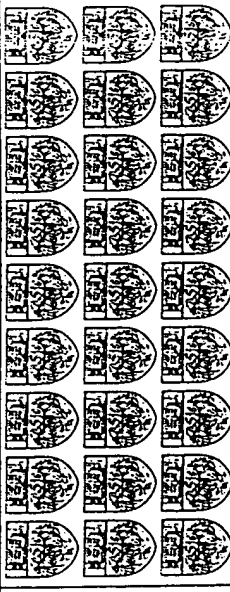
IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature
Payroll No.
Print Name
Ext.
Account Code
Date

Order placed by phone? ☐ No ☒ Yes
Darren
Order placed with (name)
Greg Sibley
Order placed by (name)
2-0294
Date
Phone #



QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1	18641-D Perked Mouse Anti-human TGF α	\$ 250	
		(Backordered 1-2 wks)	\$ 20	

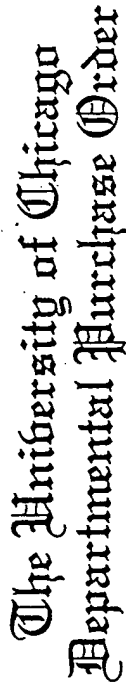
DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

ORDER TOTAL

\$270



Purchase Order Number
Z904417
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany this order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship to must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Order placed by phone? ☐ No ☒ Yes

Order placed by phone? ☐ No ☒ Yes

Order placed with (name) _____ Date _____

Virginia

Order placed by (name) _____ Phone # _____

Concey S. May *2-2284*

1

Phone #

DESCRIPTION

ITEM TOTAL

NET UNIT PRICE

\$185.00

6.25

ARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

BACK ORDERS ARE NOT ALLOWED

ORDER TOTAL

1915

J-013

Greg's Mouse Log									
Mouse #	Cage #	Group							weight
2207	AA113945	Control			18	16	11		26.4
2354	AA117027	Control ϕ			22	19	9		23.3
2356	AA117027	Control R			18	14.5	7		23.7
2357	AA117027	Control LR			17	14	10		19.6
2359	AA117028	Control L			22	18	11		20.5
2360	AA117028	Control R		18	18	18	12		24.6
2361	AA117028	Control LR			21	20	11		24.8
1710	AA105283	10 ⁷ 899-6	<10 d35		ϕ	ϕ	ϕ		24.7
2075	AA105283	10 ⁷ 899-6	<10 d42		ϕ	ϕ	ϕ		24.5
1713	AA105283	10 ⁷ 899-6	<10 d14		ϕ	ϕ	ϕ		21.5
2054	AA111331	10 ⁷ 899-6	<10 d49		ϕ	ϕ	ϕ		22.5
	105283								
1723	AA104549	10 ⁷ 3616	<10 d28 face		ϕ	ϕ	ϕ		26.5
1859	AA108972	RT Alone ϕ			15	14	10		24.8
1862	AA108972	RT Alone LR			5	5	3		25.1
1892	AA108972	RT Alone R			4	4	2		24.1
2189	AA113941	RT Alone			10	10	4.5		24.0
2190	AA113941	RT Alone			5	5	2.5		20.9
2192	AA113941	RT Alone			9	9	3		25.2
2201	AA113944	RT Alone			8	8	3		19.6
2202	AA113944	RT Alone			ϕ	ϕ	ϕ		27.0
2203	AA113944	RT Alone			11	11	4.5		20.8
2204	AA113944	RT Alone			12	11.5	7		26.6
2342	AA117024	RT Alone			14	12	5		22.1
2343	AA117024	RT Alone			11.5	9	3.5		23.5
2344	AA117024	RT Alone			12.5	9	5		22.4
2345	AA117024	RT Alone			15	8.5	5		22.9
2346	AA117025	RT Alone			15	10	6		20.0
2347	AA117025	RT Alone			11.5	11.5	7		23.9
2348	AA117025	RT Alone			16	11	7		19.4
2349	AA117025	RT Alone			12	11	5		21.7
2350	AA117026	RT Alone ϕ			16	10	6		21.3
2351	AA117026	RT Alone L			10	8	4		23.4
2352	AA117026	RT Alone R			10	10	4		21.7
2353	AA117026	RT Alone LR			13	9	1		22.1

Tuesday: Δ Clog for biohazard animals Done ✓

1863	φ	AA108971	10^7 3616+RT		4	3	2	24.1
1864	L	AA108971	10^7 3616+RT		11	10	3	25.5
1865	R	AA108971	10^7 3616+RT	<10 d38	φ	φ	φ	26.4
1866	L	AA108971	10^7 3616+RT	<10 d31	φ	φ	φ	25.6
1883	φ	AA108966	10^7 3616+RT	<10 d24	φ	φ	φ	23.1
1884	L	AA108966	10^7 3616+RT	<10 d56	φ	φ	φ	23.4
1886	L	AA108966	10^7 3616+RT	<10 d31	φ	φ	φ	19.8
2074	R	AA108966	10^7 3616+RT		11	10	3	14.7
2032	φ	AA111326	10^7 3616+RT		9	9	5	25.0
2033	L	AA111326	10^7 3616+RT		4	3	2	26.2
2035	L	AA111326	10^7 3616+RT		10	7	3	25.1
2072	R	AA111326	10^7 3616+RT	<10 d21	φ	φ	φ	25.7
2036	φ	AA111327	10^7 3616+RT		7	5	3	24.8
2037	L	AA111327	10^7 3616+RT	<10 d52	2	2	2	27.2
2039	L	AA111327	10^7 3616+RT		8	8	3	22.1
2073	R	AA111327	10^7 3616+RT		φ	φ	φ	21.7
					φ	φ	φ	
1871	φ	AA108969	10^7 899-6+RT	<10 d66	φ	φ	φ	24.8
1873	R	AA108969	10^7 899-6+RT		5.5	5	2	25.1
1874	L	AA108969	10^7 899-6+RT	<10 d52	φ	φ	φ	28.5
1880		AA108967	10^7 899-6+RT	<10 d14 L	φ	φ	φ	25.1
1881		AA108967	10^7 899-6+RT	<10 d42 R	8	9	3	27.5
1882		AA108967	10^7 899-6+RT	<10 d24 LR	φ	φ	φ	21.7
2044	φ	AA111329	10^7 899-6+RT		8	8	5	26.7
2046	R	AA111329	10^7 899-6+RT	<10 d77	φ	φ	φ	25.7
2048		AA111330	10^7 899-6+RT	<10 d38 φ	φ	φ	φ	13.5
2050		AA111330	10^7 899-6+RT	<10 d31 R	2	2	1	25.4
2051		AA111330	10^7 899-6+RT	LR	18	13	5	26.3

Greg's Mouse Log

Mouse #	Cage #	Group					weight
2207	AA113945	Control		15	16	14	
2354	AA117027	Control	SAC	23	22	13	
2356	AA117027	Control		19.5	17	11	
2357	AA117027	Control		18	15	12	
2359	AA117028	Control	SAC	23	20	15	
2360	AA117028	Control	SAC	18	17	14.5	
2361	AA117028	Control	SAC	22	23	16.5	
1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1723	AA104549	10^7 3616	<10 d28 face	0			
1859	AA108972	RT Alone	Sac'd ill	12	12	13	
1862	AA108972	RT Alone		4.5	6	4	
1892	AA108964	RT Alone		3.5	5	2.5	
2189	AA113941	RT Alone		-9.5	10	6.5	
2190	AA113941	RT Alone		5	4.5	2.5	
2192	AA113941	RT Alone		9.5	8	4	
2201	AA113944	RT Alone		8.5	7	3.5	
2202	AA113944	RT Alone		0			
2203	AA113944	RT Alone		10	9	5	
2204	AA113944	RT Alone		13	10.5	8.5	
2342	AA117024	RT Alone		12	10.5	6.5	5
2343	AA117024	RT Alone		12	10	7	
2344	AA117024	RT Alone		15	12.5	8	
2345	AA117024	RT Alone		-10.5	8.5	5.5	
2346	AA117025	RT Alone		13	11	9	
2347	AA117025	RT Alone		12	10.5	9	
2348	AA117025	RT Alone		14.5	10	8	
2349	AA117025	RT Alone		11	9.5	5	
2350	AA117026	RT Alone		-14.5	10.5	7	
2351	AA117026	RT Alone		10	8	7	
2352	AA117026	RT Alone		9.5	8	6.5	
2353	AA117026	RT Alone		13.5	8.5	7	

17
7
7
114
x18
952
1190

16
7
112
x15
160
1120

1863	AA108971	10^7 3616+RT		5	4	2.5		
1864	AA108971	10^7 3616+RT		11.5	4.5	6.5		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	0				
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT		14	10	6		
2032	AA111326	10^7 3616+RT		8	9.5	8		
2033	AA111326	10^7 3616+RT		4	3.5	2		
2035	AA111326	10^7 3616+RT		9	8	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		6	5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		7	6	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	0				
1873	AA108969	10^7 899-6+RT		6	5	4		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	8.5	10	6.5		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		8.5	7.5	6		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38 SAC	wt loss				
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT		21.5	16	10		

2209 4A113446 10^7 3616 8 6.5 7.5
 10 impl 5/3 inj 6/8 7.5 6.5 5.5
 11 10 7 6
 12 8 8.5 6

Greg's Mouse Log									
Mouse #	Cage #	Group							weight
2207	AA113945	Control		21	19	9			23.3
2354	AA117027	Control							
2356	AA117027	Control	dead						
2357	AA117027	Control	dead						
2359	AA117028	Control							
2360	AA117028	Control							
2361	AA117028	Control							
1710	AA105283	10^7 899-6	<10 d35	0					
2075	AA105283	10^7 899-6	<10 d42	0					
1713	AA105283	10^7 899-6	<10 d14	0					
2054	AA111331	10^7 899-6	<10 d49	0					
1723	AA104549	10^7 3616	<10 d28 face	0					
1859	AA108972	RT Alone							
1862	AA108972	RT Alone		5.5	6	7			25.1
1892	AA108964	RT Alone		5.5	3	2			25.3
2189	AA113941	RT Alone		9.5	11	7.5			24.8
2190	AA113941	RT Alone		3.5	3	2			20
2192	AA113941	RT Alone		8.5	8	4			24.2
2201	AA113944	RT Alone		6	7	4			22.3
2202	AA113944	RT Alone		0					27.8
2203	AA113944	RT Alone		9	10	4.5			26.5
2204	AA113944	RT Alone		14	11	8			25.5
2342	AA117024	RT Alone		14	15.5	6			23.6
2343	AA117024	RT Alone		10	9	6			23.4
2344	AA117024	RT Alone		12	10	6.5			24
2345	AA117024	RT Alone		10	12	4			23.8
2346	AA117025	RT Alone		13	9.5	7			19.4
2347	AA117025	RT Alone		12	11.5	8			23.4
2348	AA117025	RT Alone		15.5	9	8			18.4
2349	AA117025	RT Alone		13	10	7			20.7
2350	AA117026	RT Alone		14	9.5	7			21.8
2351	AA117026	RT Alone		12.5	8	6.5			22.9
2352	AA117026	RT Alone		9	8	5			23.4
2353	AA117026	RT Alone		10	8	5			24.3

1863	AA108971	10^7 3616+RT		5	4	3		23.8
1864	AA108971	10^7 3616+RT		9	8	4		26.3
1865	AA108971	10^7 3616+RT	<10 d38	0				25.8
1866	AA108971	10^7 3616+RT	<10 d31	0				25.7
1883	AA108966	10^7 3616+RT	<10 d24	0				22.8
1884	AA108966	10^7 3616+RT	<10 d56	1	1	1		21.5
1886	AA108966	10^7 3616+RT	<10 d31	0				19.6
2074	AA108966	10^7 3616+RT		19	10	7		15.8
2032	AA111326	10^7 3616+RT		8.5	8.5	7		24.3
2033	AA111326	10^7 3616+RT		3.5	3.5	2.5		26.6
2035	AA111326	10^7 3616+RT		8	9	4		24.9
2072	AA111326	10^7 3616+RT	<10 d21	0				25.9
2036	AA111327	10^7 3616+RT		4.5	5	3		22.8
2037	AA111327	10^7 3616+RT	<10 d52	0				25.9
2039	AA111327	10^7 3616+RT		7	4.5	2.5		21.2
2073	AA111327	10^7 3616+RT		0				21.1
1871	AA108969	10^7 899-6+RT	<10 d66	0				25.8
1873	AA108969	10^7 899-6+RT		4	7	3.5		26.1
1874	AA108969	10^7 899-6+RT	<10 d52	0				28.9
1880	AA108967	10^7 899-6+RT	<10 d14	0				20.4
1881	AA108967	10^7 899-6+RT	<10 d42	9	10.5	6		28.8
1882	AA108967	10^7 899-6+RT	<10 d24	0				24.3
2044	AA111329	10^7 899-6+RT		8	9.5	6		25.8
2046	AA111329	10^7 899-6+RT	<10 d77	0				25.4
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	4	2.5	1.5		26.2
2051	AA111330	10^7 899-6+RT	SOC	16	26	12		-

3616*
 2209 10 8.5 6 25.2
 2210 9 7 5 25.2
 2211 13 9.5 9.5 21.8
 2212 8.5 9 6.5 22.6

(#315B)

ARC USE ONLY

FLAGGED BY PROTOCOL

HOUSE AT: X CARLSON _____ WYLER _____ CLSC _____ FMI _____ OTHER _____

J-013

Greg's Mouse Log #2

Greg's Mouse Log #2						
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2354	DD117027	Control		None	None	
2355	DD117027	Control		None	None	
2356	DD117027	Control		None	None	
2357	DD117027	Control		None	None	
2358	DD117028	Control		None	None	
2359	DD117028	Control		None	None	
2360	DD117028	Control		None	None	
2361	DD117028	Control		None	None	
2667	AA12076	Control	13-16-13	10/6		
2668	AA12076	Control	17-10-13	10/6		
2669	AA12076	Control	16-11-13	10/6		
2670	AA12076	Control	15-11-13	10/6		
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2663	AA120077	10 ⁷ 899-6			None	
2664	AA120077	10 ⁷ 899-6			None	
2665	AA120077	10 ⁷ 899-6			None	
2666	AA120077	10 ⁷ 899-6			None	
2703	AA120078	10 ⁷ 899-6				
2704		10 ⁷ 899-6				
2705		10 ⁷ 899-6				
2706		10 ⁷ 899-6				
		10 ⁷ 899-6				
		10 ⁷ 899-6				
		10 ⁷ 899-6				
		10 ⁷ 899-6				
		10 ⁷ 899-6				
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2659	AA120091	10 ⁷ 3616			None	
2660	AA120091	10 ⁷ 3616			None	
2661	AA120091	10 ⁷ 3616			None	
2662	AA120091	10 ⁷ 3616			None	
		10 ⁷ 3616				
		10 ⁷ 3616				
		10 ⁷ 3616				
		10 ⁷ 3616				
		10 ⁷ 3616				
		10 ⁷ 3616				
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif

13 x 17 20.1
16 x 15 x 2 22.0
2 x 9
12 x 12 x 6 20.8

21x15x11	21.9
11x9x9	22.0
15x11x9	22.0
20x15x13	22.6

2346	AA117025	RT Alone				
2347	AA117025	RT Alone				
2348	AA117025	RT Alone				
2349	AA117025	RT Alone				
2350	AA117026	RT Alone				
2351	AA117026	RT Alone				
2352	AA117026	RT Alone				
2353	AA117026	RT Alone				
2687	AA12093	RT Alone				
2688	AA12093	RT Alone				
2689	AA12093	RT Alone				
2690	AA12093	RT Alone				
Mouse #	Cage #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2679	AA120094	10 ⁷ 3616+RT				
2680	AA120094	10 ⁷ 3616+RT				
2681	AA120094	10 ⁷ 3616+RT				
2682	AA120094	10 ⁷ 3616+RT				
2691	AA120089	10 ⁷ 3616+RT				
2692	AA120089	10 ⁷ 3616+RT				
2693	AA120089	10 ⁷ 3616+RT				
2694	AA120089	10 ⁷ 3616+RT				
2683	AA120086	10 ⁷ 3616+RT				
2684	AA120086	10 ⁷ 3616+RT				
2685	AA120086	10 ⁷ 3616+RT				
2686	AA120086	10 ⁷ 3616+RT				
Mouse #	Cage #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2675	AA120080	10 ⁷ 899-6+RT				
2676	AA120080	10 ⁷ 899-6+RT				
2677	AA120080	10 ⁷ 899-6+RT				
2678	AA120080	10 ⁷ 899-6+RT				
2671	AA120085	10 ⁷ 899-6+RT				
2672	AA120085	10 ⁷ 899-6+RT				
2673	AA120085	10 ⁷ 899-6+RT				
2674	AA120085	10 ⁷ 899-6+RT				
2695	AA120088	10 ⁷ 899-6+RT				
2696	AA120088	10 ⁷ 899-6+RT				
2697	AA120088	10 ⁷ 899-6+RT				
2698	AA120088	10 ⁷ 899-6+RT				

2346	AA117025	RT Alone				
2347	AA117026	RT Alone				
2348	AA117025	RT Alone				
2349	AA117025	RT Alone				
2350	AA117026	RT Alone				
2351	AA117026	RT Alone				
2352	AA117026	RT Alone				
2353	AA117026	RT Alone				
2687	AA12093	RT Alone	11-9-8 20.1			
2688	AA12093	RT Alone	12-10-8 20.2			
2689	AA12093	RT Alone	13-11-8 21.4			
2690	AA12093	RT Alone	18-1-9 23.2			
Mouse #	Case #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2679	AA11008	10^7 3616+RT	3-6-9 14.5			
2680		10^7 3616+RT	10-1-9 18.0			
2681		10^7 3616+RT	13-1-9 21.1			
2682		10^7 3616+RT	12-1-9 16.4			
2691	AA11008	10^7 3616+RT	9-8-8 14.2			
2692		10^7 3616+RT	13-9-8 22.9			
2693		10^7 3616+RT	13-11-8 18.6			
2694		10^7 3616+RT	14-11-8 14.7			
2683	AA11008	10^7 3616+RT	9-7-8 14.0			
2684		10^7 3616+RT	7-6-8 15.0			
2685		10^7 3616+RT	7-6-8 14.7			
2686		10^7 3616+RT	11-2-8 15.0			
Mouse #	Case #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2675	AA11008	10^7 899-6+RT	10-9-8 21.1			
2676		10^7 899-6+RT	13-11-8 15.2			
2677		10^7 899-6+RT	14-11-8 16.6			
2678		10^7 899-6+RT	13-11-8 21.2			
2671	AA11008	10^7 899-6+RT	7-6-8 14.0			
2672		10^7 899-6+RT	11-8-8 20.8			
2673		10^7 899-6+RT	12-11-8 13.5			
2674		10^7 899-6+RT	17-11-8 15.9			
2695	AA11008	10^7 899-6+RT	7-6-8			
2696		10^7 899-6+RT	12-11-8			
2697		10^7 899-6+RT	9-1-8			
2698		10^7 899-6+RT	1-1-8			

Greg's Mouse Log #2

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2354	DD117027	Control		None	None	
2355	DD117027	Control		None	None	
2356	DD117027	Control		None	None	
2357	DD117027	Control		None	None	
2358	DD117028	Control		None	None	
2359	DD117028	Control		None	None	
2360	DD117028	Control		None	None	
2361	DD117028	Control		None	None	
2667	AA12076	Control 8x2x5.5		7x11x9	21.7	
2668	AA12076	Control 4x6x5.5		4x10x7	21.3	
2669	AA12076	Control 5x2x6		11x11x7	21.1	
2670	AA12076	Control 6x7x5		12x10x7	21.5	

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2663	AA120077	10^7 899-6			None	21x17x11 24.5
2664	AA120077	10^7 899-6			None	11x9x8 22.5
2665	AA120077	10^7 899-6			None	8x6x3 21
2666	AA120077	10^7 899-6			None	10x9x7 21.8
2703		10^7 899-6				4/3
2704		10^7 899-6				
2705		10^7 899-6				
2706		10^7 899-6				
		10^7 899-6				
		10^7 899-6				
		10^7 899-6				
		10^7 899-6				
		10^7 899-6				

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2659	AA120091	10^7 3616			None	8x7x5 21.6
2660	AA120091	10^7 3616			None	14x11x7 21.3
2661	AA120091	10^7 3616			None	11x8x5 22.1
2662	AA120091	10^7 3616			None	13x8x5 21.
		10^7 3616				
		10^7 3616				
		10^7 3616				
		10^7 3616				
		10^7 3616				
		10^7 3616				
		10^7 3616				
		10^7 3616				

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
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16x12x9
9x8x7
9x8x5
11x9x5
11x7x6
11x8x7
12x11x9
13x9x7

10x9x7
11x9x6
11x9x6
10x7x4

21x17x11 24.5
11x9x8 22.5
8x6x3 21
10x9x7 21.8
4/3

8x7x5 21.6
14x11x7 21.3
11x8x5 22.1
13x8x5 21.

Greg's Mouse Log #2

[illegible]

20 Gy
25 Gy day 2

Greg's Mouse Log

Mouse #	Group				weight			weight
1710	10*7 899-8	0	0	0	0	0	0	20.0
2075	10*7 899-8	0	0	0	0	0	0	20.0
1713	10*7 899-8	0	0	0	0	0	0	18.9
2054	10*7 899-8	0	0	0	0	0	0	20.9
1723	10*7 3818	0	0	0	0	0	0	20.5
1862	RT Alone	11	7	4	11	8	6	26.5
1892	RT Alone	5	4	3	4	4	3	20.0
2189	RT Alone	10	10	7	11	10	8	25.5
2190	RT Alone	2.5	2	1.5	1.0	1.0	1.0	21.9
2192	RT Alone	0	0	1.5	0	5	1.5	21.5
2201	RT Alone	4	4	2.5	3	1	2	20.5
2202	RT Alone	0	0	0	0	0	0	20.5
2203	RT Alone	7	8	5	9	3	4	24.1
2204	RT Alone	20	20	10	21	21	13	29.3
2342	RT Alone	18	17	9	18	17	10	25.8
2343	RT Alone	13	12	8	13	12	8	25.0
2344	RT Alone	14	13	7	12	12	9	24.7
2345	RT Alone	10	9	5	8	7	3	25.1
2346	RT Alone	11	12.5	9	12	10	7	23.1
2347	RT Alone	13.5	13.5	11.5	15	15	12	24.3
2348	RT Alone	11	16.5	12	13	18	11	20.1
2349	RT Alone	11	11	5.5	14	11	8	22.1
2350	RT Alone	13	9.75	8	14	10	8	24.0
2351	RT Alone	0.5	9.5	0	0	0	0	24.7
2352	RT Alone	9	9	6.25	10	8	6	24.1
2353	RT Alone	9	13	0	11	7	7	22.8
1863	10*7 3818+RT	3	3	3	4	3	2	25.3
1864	10*7 3818+RT	8	7	1.5	0	0	1.5	26.8
1865	10*7 3818+RT	0	0	0	0	0	0	22.1
1866	10*7 3818+RT	0	0	0	0	0	0	24.6
1883	10*7 3818+RT	0	0	0	0	0	0	21.2
1884	10*7 3818+RT	0	0	0	0	0	0	24.2
1886	10*7 3818+RT	0	0	0	0	0	0	24.2
2032	10*7 3818+RT	12	10	7	11	9	7	25.8
2033	10*7 3818+RT	4	4	3	3	3	2.0	22.0
2035	10*7 3818+RT	0	6	5	0	5	3	24.8
2072	10*7 3818+RT	0	0	0	0	0	0	21.0
2036	10*7 3818+RT	5.5	5	2.5	0.5	0.1	2	22.2
2037	10*7 3818+RT	0	0	0	0	0	0	21.1
2039	10*7 3818+RT	6.5	5	1.5	0	1	1.5	19.7
2073	10*7 3818+RT	0	0	0	0	0	0	21.9
1871	10*7 899-6+RT	0	0	0	0	0	0	25.8
1873	10*7 899-6+RT	0	0	0	0	0	0	26.1
1874	10*7 899-6+RT	0	0	0	0	0	0	21.2
1880	10*7 899-6+RT	0	0	0	0	0	0	24.5
1881	10*7 899-6+RT	15	12	8	16	13	10	25.7
1882	10*7 899-6+RT	0	0	0	0	0	0	19.7
2044	10*7 899-6+RT	15	12	7	14	12	7	22.0
2046	10*7 899-6+RT	0	0	0	0	0	0	26.9
2050	10*7 899-6+RT	2	2	1.5	0	0	0	22.4

29.5

23.3

2346	AA117025	RT Alone					
2347	AA117025	RT Alone					
2348	AA117025	RT Alone					
2349	AA117025	RT Alone					
2350	AA117026	RT Alone					
2351	AA117026	RT Alone					
2352	AA117026	RT Alone					
2353	AA117026	RT Alone					
2687	AA11703	RT Alone	10 x 8 x 7		12 x 9 x 7		
2688		RT Alone	11 x 9 x 6		12 x 10 x 8		
2689		RT Alone	10 x 7 x 6		12 x 10 x 8		
2690		RT Alone	9 x 7 x 5		15 x 10 x 8		
2691		10^7 3616+RT	12 x 8 x 7		12 x 11 x 8		
2692		10^7 3616+RT			12 x 11 x 8		
2693		10^7 3616+RT			12 x 9 x 7		
2694		10^7 3616+RT			11 x 10 x 8		
2695		10^7 3616+RT			12 x 10 x 8		
2696		10^7 3616+RT			11 x 9 x 8		
2697		10^7 3616+RT			13 x 10 x 9		
2698		10^7 3616+RT			14 x 11 x 10		
2699		10^7 3616+RT			9 x 8 x 8		
2700		10^7 3616+RT			11 x 9 x 7		
2701		10^7 3616+RT			10 x 10 x 8		
2702		10^7 3616+RT			11 x 7 x 7		
2675		10^7 899-6+RT			12 x 11 x 8		
2676		10^7 899-6+RT			13 x 11 x 8		
2677		10^7 899-6+RT			11 x 10 x 9		
2678		10^7 899-6+RT			12 x 10 x 9		
2679		10^7 899-6+RT			11 x 7 x 7		
2680		10^7 899-6+RT			12 x 9 x 8		
2681		10^7 899-6+RT			12 x 10 x 9		
2682		10^7 899-6+RT			15 x 12 x 10		
		10^7 899-6+RT					
		10^7 899-6+RT					
		10^7 899-6+RT					
		10^7 899-6+RT					

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Table

Greg's Mouse Log

Mouse #	Group				weight					weight
1710	10^7 899-6	0								
2075	10^7 899-6	0								
1713	10^7 899-6	0								
2054	10^7 899-6	0								
1723	10^7 3616	0	0	0						
1862	RT Alone	9	8	7						
1892	RT Alone	4	4	2						
2189	RT Alone	9	9	7						
2190	RT Alone	0	0	0						
2192	RT Alone	7	7	0.3						
2201	RT Alone	4	3	2						
2202	RT Alone	0	0	0						
2203	RT Alone	7	6	2						
2204	RT Alone	22	22	17						
2342	RT Alone	17	17	10						
2343	RT Alone	11	10	7						
2344	RT Alone	13	11	7						
2345	RT Alone	7	5	1.5						
2346	RT Alone	12	9	7						
2347	RT Alone	17	14	9						
2348	RT Alone	14	12	10						
2349	RT Alone	13	10	7						
2350	RT Alone	14	10	7						
2351	RT Alone	10	8	7						
2352	RT Alone	9	8	6						
2353	RT Alone	11	9	7						
1863	10^7 3616+RT	3	2	2						
1864	10^7 3616+RT	0								
1865	10^7 3616+RT	0								
1866	10^7 3616+RT	0	0	0						
1883	10^7 3616+RT	0	0	0						
1884	10^7 3616+RT									
1886	10^7 3616+RT	0	0	0						
2032	10^7 3616+RT	12	10	8						
2033	10^7 3616+RT	2	2	1.5						
2035	10^7 3616+RT	8	5	2						
2072	10^7 3616+RT	0	0	0						
2036	10^7 3616+RT	5	4	2						
2037	10^7 3616+RT	0	0	0						
2039	10^7 3616+RT	2	2	1.5						
2073	10^7 3616+RT	0	0	0						
1871	10^7 899-6+RT	0	0	0						
1873	10^7 899-6+RT	0	0	0						
1874	10^7 899-6+RT	0	0	0						
1880	10^7 899-6+RT	0	0	0						
1881	10^7 899-6+RT	16	14	10						
1882	10^7 899-6+RT	0	0	0						
2044	10^7 899-6+RT	14	12	10						
2046	10^7 899-6+RT	0	0	0						
2050	10^7 899-6+RT	2.5	2	1.5						

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Greg's Mouse Log

Greg's Mouse Log						
Mouse #	Group				weight	weight
1710	10^7 899-6	0	-	-	26.7	
2075	10^7 899-6	0	0	0	26.7	
1713	10^7 899-6	0	-	-	29.0	
2054	10^7 899-6	0	-	-	25.1	
1723	10^7 3616	0	-	-	25.7	
1862	RT Alone	11	8	7	26.1	
1882	RT Alone	3	3	2	26.1	
2189	RT Alone	0	7	5	25.2	
2190	RT Alone	0	-	-	25.2	
2192	RT Alone	4	4	1.5	26.0	
2201	RT Alone	3	5	1.5	27.3	
2202	RT Alone	0	-	-	24.6	
2203	RT Alone	5	4	1.5	25.5	
2204	RT Alone	5	4	1.5	25.5	
2342	RT Alone	17	17	12	25.0	
2343	RT Alone	12	11	8	24.3	
2344	RT Alone	12	9	8	24.7	
2345	RT Alone	7	3	1.5	25.5	
2346	RT Alone	12	10	7	23.4	
2347	RT Alone	18	15	11	26.3	
2348	RT Alone	15	13	4	20.4	
2349	RT Alone	14	10	8	21.2	
2350	RT Alone	13	10	8	25.4	
2351	RT Alone	8	7	6	25.1	
2352	RT Alone	4	8	5	27.6	
2353	RT Alone	11	7	4	25.5	
1863	10^7 3616+RT	3	3	1	24.7	
1864	10^7 3616+RT	0	-	-	24.7	
1865	10^7 3616+RT	6	-	-	24.3	
1866	10^7 3616+RT	0	-	-	26.4	
1883	10^7 3616+RT	0	-	-	24.5	
1884	10^7 3616+RT	0	-	-	25.4	
2032	10^7 3616+RT	12	9	8	25.7	
2033	10^7 3616+RT	7	2	21	26.6	
2035	10^7 3616+RT	7	2	1	24.7	
2072	10^7 3616+RT	0	-	-	23.9	
2036	10^7 3616+RT	3	3	2	21.4	
2037	10^7 3616+RT	1	1	1	26.4	
2039	10^7 3616+RT	6	-	-	19.0	
2073	10^7 3616+RT	0	-	-	21.3	
1871	10^7 899-6+RT	0	-	-	26.4	
1873	10^7 899-6+RT	0	-	-	27.2	
1874	10^7 899-6+RT	0	-	-	25.6	
1880	10^7 899-6+RT	0	-	-	19.8	
1881	10^7 899-6+RT	15	13	11	27.2	
1882	10^7 899-6+RT	0	-	-	24.0	
2044	10^7 899-6+RT	11	10	7	26.4	
2046	10^7 899-6+RT	0	-	-	27.0	
2050	10^7 899-6+RT	1	1	1	27.0	

Greg's Mouse Log									
Mouse #	Cage #	Group							weight
2207	AA113945	Control							
2354	AA117027	Control							
2356	AA117027	Control							
2357	AA117027	Control							
2359	AA117028	Control							
2360	AA117028	Control							
2361	AA117028	Control							
1710	AA105283	10^7 899-6	<10 d35	0					
2075	AA105283	10^7 899-6	<10 d42	0					
1713	AA105283	10^7 899-6	<10 d14	0					
2054	AA111331	10^7 899-6	<10 d49	0					
1723	AA104549	10^7 3616	<10 d28 face	0					
1859	AA108972	RT Alone							
1862	AA108972	RT Alone		5	6	4.5			
1892	AA108964	RT Alone		4.5	3	2.5			
2189	AA113941	RT Alone		10	13	6			
2190	AA113941	RT Alone		4	3	1.5			
2192	AA113941	RT Alone		8	7	4			
2201	AA113944	RT Alone		6	7	4			
2202	AA113944	RT Alone		0					
2203	AA113944	RT Alone		9	9	4			
2204	AA113944	RT Alone		13.5	11	9			
2342	AA117024	RT Alone		8	13.5	5.5			
2343	AA117024	RT Alone		10	9	6			
2344	AA117024	RT Alone		11.5	9	6.5			
2345	AA117024	RT Alone		13	14.5	9			
2346	AA117025	RT Alone		13	11	8			
2347	AA117025	RT Alone		10.5	10.5	7			
2348	AA117025	RT Alone		15	9.5	8.5			
2349	AA117025	RT Alone		10	14	7.5			
2350	AA117026	RT Alone		14	10.5	7			
2351	AA117026	RT Alone		10	8	6			
2352	AA117026	RT Alone		9.5	8.5	6			
2353	AA117026	RT Alone		12	9	6.7			

~~3016~~
~~2209~~
~~2210~~
~~2211~~
~~2212~~

1863	AA108971	10^7 3616+RT		4.5	3	2.5		
1864	AA108971	10^7 3616+RT		8.5	6	3		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	-1.5	1.5	1.5		
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT	sac wt loss	-21.4	12.5	7		
2032	AA111326	10^7 3616+RT		-8	8	6		
2033	AA111326	10^7 3616+RT		3	2	1.5		
2035	AA111326	10^7 3616+RT		8.5	8.5	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		5	4.5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		6	4.5	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	6	4.5	3		
1873	AA108969	10^7 899-6+RT		4.5	5.5	3.5		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	10	10.5	6		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		9	8	9		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT						

Greg's Mouse Log									
Mouse #	Cage #	Group							weight
2207	AA113945	Control							
2354	AA117027	Control							
2356	AA117027	Control							
2357	AA117027	Control							
2359	AA117028	Control							
2360	AA117028	Control							
2361	AA117028	Control							
1710	AA105283	10^7 899-6	<10 d35						24.9
2075	AA105283	10^7 899-6	<10 d42						24.8
1713	AA105283	10^7 899-6	<10 d14						22.4
2054	AA111331	10^7 899-6	<10 d49						23.7
1723	AA104549	10^7 3616	<10 d28 face	10					26.7
1859	AA108972	RT Alone							
1862	AA108972	RT Alone		5	7	4.5			26.3
1892	AA108964	RT Alone		8.5	3.5	1.5			25.7
2189	AA113941	RT Alone		10	13	9			25.7
2190	AA113941	RT Alone		4	3	2			21.7
2192	AA113941	RT Alone		8	7.5	2.5			24.7
2201	AA113944	RT Alone		6	6	3			26.4
2202	AA113944	RT Alone		0					26.2
2203	AA113944	RT Alone		9.5	9	4			23.1
2204	AA113944	RT Alone		16	12	8			26.2
2342	AA117024	RT Alone		13.5	13.5	8			24
2343	AA117024	RT Alone		11	9	7			24.3
2344	AA117024	RT Alone		11.5	10	5			24.0
2345	AA117024	RT Alone		9.5	7	3			24.9
2346	AA117025	RT Alone		12	11	8			26.0
2347	AA117025	RT Alone		12	11.5	7			25.8
2348	AA117025	RT Alone		14	9.5	9			18.8
2349	AA117025	RT Alone		11	12	6.5			20.3
2350	AA117026	RT Alone		10	8	5.5			24.0
2351	AA117026	RT Alone		9.5	15	7			27.8
2352	AA117026	RT Alone		9	8.5	5			23.5
2353	AA117026	RT Alone		12.5	8	7			22

1863	AA108971	10^7 3616+RT		4	3	2	24.9
1864	AA108971	10^7 3616+RT		7	4.5	2.5	27.0
1865	AA108971	10^7 3616+RT	<10 d38	0			27.1
1866	AA108971	10^7 3616+RT	<10 d31	0			26.3
1883	AA108966	10^7 3616+RT	<10 d24	0			22.6
1884	AA108966	10^7 3616+RT	<10 d56	0			21.2
1886	AA108966	10^7 3616+RT	<10 d31	0			20.8
2074	AA108966	10^7 3616+RT					
2032	AA111326	10^7 3616+RT		9.5	10	7	25.7
2033	AA111326	10^7 3616+RT		3.5	4.5	2.5	26.3
2035	AA111326	10^7 3616+RT		7	7.5	4	24.7
2072	AA111326	10^7 3616+RT	<10 d21	6.5	5.5	3	26.0
2036	AA111327	10^7 3616+RT		5	4.5	3	23.2
2037	AA111327	10^7 3616+RT	<10 d52	0			27
2039	AA111327	10^7 3616+RT		5.5	4	2.5	20.8
2073	AA111327	10^7 3616+RT		0			22
1871	AA108969	10^7 899-6+RT	<10 d66	5	5	3	26.5
1873	AA108969	10^7 899-6+RT		6	5	3	26.3
1874	AA108969	10^7 899-6+RT	<10 d52	0			28.1
1880	AA108967	10^7 899-6+RT	<10 d14	0			19.8
1881	AA108967	10^7 899-6+RT	<10 d42	9.5	13	8	30
1882	AA108967	10^7 899-6+RT	<10 d24	0			24
2044	AA111329	10^7 899-6+RT		12.5	10	9.5	27.4
2046	AA111329	10^7 899-6+RT	<10 d77	0			25.4
2048	AA111330	10^7 899-6+RT	<10 d38				
2050	AA111330	10^7 899-6+RT	<10 d31	2.5	2.5	1	26.4
2051	AA111330	10^7 899-6+RT					

Greg's Mouse Log									
Mouse #	Group							weight	weight
1710	10^7 899-6								
2075	10^7 899-6								
1713	10^7 899-6								
2054	10^7 899-6								
1723	10^7 3616	0							
1862	RT Alone	8	6	5			8	6	5
1892	RT Alone	7	3	2			3	3	2
2189	RT Alone	14	10	9			11	12.5	8
2190	RT Alone	2	2	1			0		
2192	RT Alone	6	5	3			6	7.5	2
2201	RT Alone	0					-5.5	5	3
2202	RT Alone	8	8	5			0		
2203	RT Alone	6	6	4			8	8.5	4.5
2204	RT Alone	17	13	11			-17.5	16	14
2342	RT Alone						16.5	14	9
2343	RT Alone						10.5	10	6
2344	RT Alone						11	11	7
2345	RT Alone						-14	7.5	4
2346	RT Alone	13	12	9			-13	11	8
2347	RT Alone	15	14	7			-12.5	14	6.5
2348	RT Alone	16	11	7			15	9.5	7
2349	RT Alone	12	10	8			10	15	6
2350	RT Alone	12	8	4			10	14.5	8
2351	RT Alone	7	3	3			9	7.5	6
2352	RT Alone	5	5	2			9	8	5
2353	RT Alone	9	6	6			-12.5	9	6.5
1863	10^7 3616+RT	9	4	2					
1864	10^7 3616+RT	8	7	2					
1865	10^7 3616+RT	0							
1866	10^7 3616+RT	0							
1883	10^7 3616+RT	0							
1884	10^7 3616+RT	0							
1886	10^7 3616+RT	0							
2032	10^7 3616+RT								
2033	10^7 3616+RT								
2035	10^7 3616+RT								
2072	10^7 3616+RT								
2036	10^7 3616+RT								
2037	10^7 3616+RT								
2039	10^7 3616+RT								
2073	10^7 3616+RT								
1871	10^7 899-6+RT								
1873	10^7 899-6+RT								
1874	10^7 899-6+RT								
1880	10^7 899-6+RT								
1881	10^7 899-6+RT								
1882	10^7 899-6+RT								
2044	10^7 899-6+RT								
2046	10^7 899-6+RT								
2050	10^7 899-6+RT								

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Greg's Mouse Log									
Mouse #	Group							weight	weight
1710	10^7 899-6	0							
2075	10^7 899-6	0							
1713	10^7 899-6	0							
2054	10^7 899-6	0							
1723	10^7 3616								
1862	RT Alone								
1892	RT Alone								
2189	RT Alone								
2190	RT Alone								
2192	RT Alone								
2201	RT Alone								
2202	RT Alone								
2203	RT Alone								
2204	RT Alone								
2342	RT Alone	-16.5	16	7					
2343	RT Alone	-11.5	8	5					
2344	RT Alone	-11.5	11	6					
2345	RT Alone	-10	7	4					
2346	RT Alone								
2347	RT Alone								
2348	RT Alone								
2349	RT Alone								
2350	RT Alone								
2351	RT Alone								
2352	RT Alone								
2353	RT Alone								
1863	10^7 3616+RT								
1864	10^7 3616+RT								
1865	10^7 3616+RT								
1866	10^7 3616+RT								
1883	10^7 3616+RT								
1884	10^7 3616+RT								
1886	10^7 3616+RT								
2032	10^7 3616+RT	10	11	5					
2033	10^7 3616+RT	3	3	2					
2035	10^7 3616+RT	8	7	3.5					
2072	10^7 3616+RT	0							
2036	10^7 3616+RT	-5.5	4	3					
2037	10^7 3616+RT	-0							
2039	10^7 3616+RT	-4.5	3	2					
2073	10^7 3616+RT	0							
1871	10^7 899-6+RT	0							
1873	10^7 899-6+RT	7	6	3.5					
1874	10^7 899-6+RT	0							
1880	10^7 899-6+RT	0							
1881	10^7 899-6+RT	0							
1882	10^7 899-6+RT	11.5	14	6					
2044	10^7 899-6+RT	14	12	11					
2046	10^7 899-6+RT	0							
2050	10^7 899-6+RT	0							

Greg's Mouse Log									
Mouse #	Group					weight			weight
1710	10^7 899-6	0				25.8			
2075	10^7 899-6	0				25.2			
1713	10^7 899-6	0				22.7			
2054	10^7 899-6	0				24.3			
1723	10^7 3616	0				26.3			
1862	RT Alone	3	7	4		28.2			
1892	RT Alone	4	4	2		22.9			
2189	RT Alone	14	11	10		25.3			
2190	RT Alone	0				22.0			
2192	RT Alone	6	6	6		25.0			
2201	RT Alone	5	5	14		28.0			
2202	RT Alone	0				27.9			
2203	RT Alone	9	9	6		23.3			
2204	RT Alone	18	17	10		27.6			
2342	RT Alone	13	12	6		25.0			
2343	RT Alone	12	7	7		26.3			
2344	RT Alone	19	15	9		25.3			
2345	RT Alone	10	8	5		25.7			
2346	RT Alone	11	11	8		22.8			
2347	RT Alone	15	14	9		26.0			
2348	RT Alone	16	9	7		18.3			
2349	RT Alone	16	16	6		22.2			
2350	RT Alone	15	11	6		25.5			
2351	RT Alone	9	8	5		24.2			
2352	RT Alone	9	9	4		24.7			
2353	RT Alone	11	12	6		27.3			
1863	10^7 3616+RT	4	4	2		25.3			
1864	10^7 3616+RT	9	8	1.5		25.6			
1865	10^7 3616+RT	0				27.9			
1866	10^7 3616+RT	0				26.7			
1883	10^7 3616+RT	0				23.4			
1884	10^7 3616+RT	0				19.5			
1886	10^7 3616+RT	0				22.6			
2032	10^7 3616+RT	0				26.0			
2033	10^7 3616+RT	4	3	1.5		26.5			
2035	10^7 3616+RT	7	6	4		24.7			
2072	10^7 3616+RT	9	7	5		26.8			
2036	10^7 3616+RT	6	5	1.5		23.9			
2037	10^7 3616+RT	0				27.3			
2039	10^7 3616+RT	5	5	1.5		19.9			
2073	10^7 3616+RT	0				22.5			
871	10^7 899-6+RT	0				25.9			
873	10^7 899-6+RT	7	6	1.5		26.1			
874	10^7 899-6+RT	0				27.0			
880	10^7 899-6+RT	0				19.8			
881	10^7 899-6+RT	15	12	7		30.1			
882	10^7 899-6+RT	0				23.9			
044	10^7 899-6+RT	14	14	7		28.2			
046	10^7 899-6+RT	0				26.3			
050	10^7 899-6+RT	9				26.4			

11 1000

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24.5
23.2
22.10
28.7
21.4
17.1
23.1
A-3

1087x5

→ SAC

11 x 7 x 4

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2189	RT Alone	6x6x3	7x6x3
2190	RT Alone	0x-	0
2192	RT Alone	2x2x1	1x1x1
2201	RT Alone	2x2x1.5	3x3x1.5
2202	RT Alone	0x-	0
2203	RT Alone	4x4x3	4x3x3
2204	RT Alone		SAC
2342	RT Alone	11x10x3	11x9x3
2343	RT Alone	12x10x3	11x10x7
2344	RT Alone	13x12x3	11x11x12
2345	RT Alone	11x11x3	6x3x1.5
2346	RT Alone	12x10x3	8x7x5
2347	RT Alone	13x12x3	17x16x11
2348	RT Alone	13x12x3	16x14x9
2349	RT Alone	14x16x10	14x16x8
2350	RT Alone	12x14x8	12x9x6
2351	RT Alone	8x6x5	9x7x4
2352	RT Alone	9x8x4	8x8x3
2353	RT Alone	11x6x5	9x6x4
2687	RT Alone	12x10x3	11x9x7
2688	RT Alone	13x11x9	14x14x8
2689	RT Alone	13x8x8	14x8x7
2690	RT Alone	10x7x5	9x7x6
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
* 2667	Control	19x16x15	
2668	Control	16x15x12	16x14x13
2669	Control	16x15x11	18x16x13
2670	Control	22x15x12	22x15x10

26.7

23.4

25.9

28.2

29.9

23.5

42.3

24.4

24.0

24.1

23.4

26.3

21.4

17.9

26.1

21.9

26.0

25.1

21.0

23.9

24.5

12x11x8 21.8

deceased

18.5

23.0

Data Sheet

2659	3616	14x11x9	15x15x15
2660	3616	22x18x13	28x19x11 ⇒ SAC
2661	3616	19x15x11	18x16x7
2662	3616	23x15x15	22x17x12 ⇒ SAC
2701	3616	13x10x7	
2706	3616	12x9x6	
2709	3616	11x9x7	
2710	3616	13x11x8	
2719	3616	9x8x6	
2720	3616	7x8x7	
2721	3616	7x8x7	
2722	3616	10x8x6	

AA12008

AA12008

Amended

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2189	RT Alone	9 x 5 x 1.5	25.8
2190	RT Alone	0	23.9
2192	RT Alone	3 x 3 x 2	25.9
2201	RT Alone	5 x 2 x 1.5	26.1
2202	RT Alone	0	29.3
2203	RT Alone	4 x 3 x 1.5	24.2
2204	SAC	RT Alone	
2342	RT Alone	11 x 9 x 4	21.9
2343	RT Alone	11 x 12 x 6	21.1
2344	RT Alone	22 x 10 x 10	24.0
2345	RT Alone	6 x 3 x 1.5	24.3
2346	RT Alone	7 x 8 x 5	23.5
2347	RT Alone	16 x 16 x 10	25.2
2348	RT Alone	5 x 15 x 10	21.0
2349	RT Alone	Area 7/10/15	
2350	RT Alone	8 x 8 x 3	24.3
2351	RT Alone	12 x 9 x 5	27.1
2352	RT Alone	8 x 8 x 3	25.7
2353	RT Alone	9 x 6 x 4	25.3
2687	RT Alone	12 x 9 x 6	22.0
2688	RT Alone	12 x 10 x 6	23.5
2689	RT Alone	12 x 8 x 5	24.7
2690	RT Alone	9 x 7 x 6	25.1
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
2667	SAC	Control	
2668	Control		
2669	Control	19 x 14 x 14	22.7
2670	Control	21 x 13 x 8	17.4

Data Sheet

		15.1	1.1
2679	10^7 3636RT	5 x 4 x 1.5	10.4
2680	10^7 3616RT	7 x 4 x 1.5	13.1
2681	10^7 3636RT	11 x 4 x 5	21.2
2682	10^7 3616RT	13 x 8 x 8	20.0
2691	10^7 3636RT	7 x 6 x 4	20.5
2692	10^7 3616RT	9 x 8 x 6	23.1
2693	10^7 3636RT	12 x 10 x 3	22.6
2694	10^7 3616RT	13 x 9 x 7	24.1
2683	10^7 3616RT	7 x 6 x 4	23.2
2684	10^7 3636RT	0	19.0
2685	10^7 3616RT	6 x 3 x 1.5	23.4
2686	10^7 3616RT	10 x 8 x 4	18.9
2675	10^7 8996RT	11 x 8 x 5	25.2
2676	10^7 8996RT	13 x 12 x 7	17.3
2677	10^7 8996RT	12 x 9 x 5	19.1
2678	10^7 8996RT	12 x 10 x 6	25.8
2671	10^7 8996RT	7 x 7 x 4	22.2
2672	10^7 8996RT	8 x 6 x 4	25.1
2673	10^7 8996RT	14 x 11 x 6	20.1
2674	10^7 8996RT	11 x 8 x 4	14.4
2695	10^7 8996RT	10 x 9 x 6	22.7
2696	10^7 8996RT	17 x 13 x 9	19.2
2697	10^7 8996RT	10 x 10 x 6	24.9
2698	10^7 8996RT	12 x 10 x 8	17.1
2663	899-6	16 x 15 x 7	22.4
2664	SAC 899-6		
2665	899-6	21 x 17 x 13	24.3
SAC 2666	899-6		
2703	899-6	13 x 8 x 5	25.2
2704	899-6	12 x 11 x 4	27.3
2705	899-6	21 x 15 x 9	26.3
2706	899-6	19 x 13 x 6	21.0
	899-6		
	899-6		
	899-6		
	899-6		

Data Sheet

2659	3616	20x20x14	24.8
2660 SAC	3616		
2661	3616	21x14x12	23.2
2662 SAC	3616		
2707	3616		
2708	3616		
2709	3616		
2710	3616		
2719	3616		
2720	3616		
2721	3616		
2722	3616		

Data Sheet

Greg's Mouse	Data			
Mouset#	Group	Measurements	Weight	
2189	RT Alone	8x8x1.5	5x5x1.5	28.3
2190	RT Alone	Ø	Ø	25.9
2192	RT Alone	Ø	Ø	23.3
2201	RT Alone	5x3x1.5	5x3x1.5	29.8
2202	RT Alone	Ø	Ø	29.1
2203	RT Alone	5x4x1.5	6x4x1.5	23.3
2204	SAC	RT Alone		
2342	RT Alone	10x17x12	22x17x12	24.6
2343	RT Alone	12x10x5	12x10x6	24.7
2344	RT Alone	Ø	Ø	23.1
2345	RT Alone	Ø	Ø	24.7
2346	RT Alone	11x7x3	8x7x3	25.1
2347	RT Alone	15x15x10	17x15x10	26.0
2348	RT Alone	15x15x9	14x11x9	20.8
SAC	2349	RT Alone		
2350	RT Alone	12x9x6	10x9x5	27.2
2351	RT Alone	10x8x4	10x7x3	25.5
2352	RT Alone	9x9x1.5	9x9x1.5	26.2
2353	RT Alone	10x7x2	9x5x4	25.7
2687	RT Alone	11x9x6	11x10x4	28.6
2688	RT Alone	12x9x5	12x9x4	26.3
2689	RT Alone	11x9x8	12x9x4	24.3
2690	RT Alone	10x8x4	9x7x4	27.3
2354	Control			
2355	Control			
2356	Control			
2357	Control			
2358	Control			
2359	Control			
2360	Control			
2361	Control			
2667	SAC	Control		
2668	Control			
2669	Control			
2670	Control			

Data Sheet

2679	10^7 3636RT	Ø	Ø	23.8
2680	10^7 3616RT	4x2x1.5	Ø	13.0
2681	10^7 3636RT	10x9x3	10x8x3	23.7
2682	10^7 3616RT	13x9x7	12x8x6	20.9
2691	10^7 3636RT	6x5x2	6x4x2	24.3
2692	10^7 3616RT	11x8x5	11x9x5	25.5
2693	10^7 3636RT	11x11x4	10x8x4	29.0
2694	10^7 3616RT	13x10x6	10x9x3	18.6
2683	10^7 3616RT	9x7x2	6x5x1.5	23.6
2684	10^7 3636RT	Ø	Ø	28.2
2685	10^7 3616RT	3x1.5x1.5	Ø	23.2
2686	10^7 3616RT	7x6x4	7x5x3	21.4
2675	10^7 8996RT	7x7x4	11x11x5	19.6
2676	10^7 8996RT	12x11x6	7x5x2	27.4
2677	10^7 8996RT	12x9x3	10x7x3	28.2
2678	10^7 8996RT	10x10x4	9x9x4	28.4
2671	10^7 8996RT	5x4x4	5x4x2	23.0
2672	10^7 8996RT	5x3x1.5	5x3x1.5	26.4
2673	10^7 8996RT	11x9x7	9x7x4	20.3
2674	10^7 8996RT	8x6x2	6x5x1.5	17.4
2695	10^7 8996RT	10x9x5	8x5x3	21.6
2696	10^7 8996RT	10x9x6	12x9x5	17.3
2697	10^7 8996RT	11x12x6	8x7x5	26.6
2698	10^7 8996RT	12x8x6	16x13x7	21.1
2663	899-6	15x12x6	16x10x4	20.4
2664	SAC 899-6			
SAC	2665 899-6	23x19x14		SAC
SAC	2666 899-6			
2703	899-6	17x12x4	17x14x9	26.2
2704	899-6	15x13x6	19x5x6	29.6
2705	899-6	19x17x9	11x15x10	21.0
2706	899-6	20x15x8	21x15x8	27.4
2707	899-6			
2708	899-6			
2709	899-6			
2710	899-6			
2711	899-6			
2712	899-6			
2713	899-6			
2714	899-6			
2715	899-6			
2716	899-6			
2717	899-6			
2718	899-6			
2719	899-6			
2720	899-6			
2721	899-6			
2722	899-6			
2723	899-6			
2724	899-6			
2725	899-6			
2726	899-6			
2727	899-6			
2728	899-6			
2729	899-6			
2730	899-6			
2731	899-6			
2732	899-6			
2733	899-6			
2734	899-6			
2735	899-6			
2736	899-6			
2737	899-6			
2738	899-6			
2739	899-6			
2740	899-6			
2741	899-6			
2742	899-6			
2743	899-6			
2744	899-6			
2745	899-6			
2746	899-6			
2747	899-6			
2748	899-6			
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2750	899-6			
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2758	899-6			
2759	899-6			
2760	899-6			
2761	899-6			
2762	899-6			
2763	899-6			
2764	899-6			
2765	899-6			
2766	899-6			
2767	899-6			
2768	899-6			
2769	899-6			
2770	899-6			
2771	899-6			
2772	899-6			
2773	899-6			
2774	899-6			
2775	899-6			
2776	899-6			
2777	899-6			
2778	899-6			
2779	899-6			
2780	899-6			
2781	899-6			
2782	899-6			
2783	899-6			
2784	899-6			
2785	899-6			
2786	899-6			
2787	899-6			
2788	899-6			
2789	899-6			
2790	899-6			
2791	899-6			
2792	899-6			
2793	899-6			
2794	899-6			
2795	899-6			
2796	899-6			
2797	899-6			
2798	899-6			
2799	899-6			
2800	899-6			

Data Sheet

MC 2659	3616	21x21x15		
2660 SAG	3616			
MC 2661	3616	25x11x13		
2662 SAG	3616			
2707	3616	11x9x7	11x9x7	23.8
2708	3616	12x10x6	12x10x5	22.8
2709	3616	14x12x7	14x13x10	25.0
2710	3616	13x11x7	14x11x8	25.0
2719	3616	7x7x4	6x5x15	22.0
2720	3616	11x9x5	8x6x3	25.1
2721	3616	7x6x4	13x8x6	23.3
2722	3616	9x8x4	9x6x4	22.7

Data Sheet

Greg's Mouse	Data			
Mouse #	Group	Measurements	Weight	
2189	RT Alone	Ø	Ø	24.5
2190	RT Alone	Ø	Ø	22.4
2192	RT Alone	Ø	Ø	25.0
2201	RT Alone	Ø	Ø	28.0
2202	RT Alone	5x3x1.5	Ø	28.4
2203	RT Alone	4x4x1.5	4x4x1.5	22.9
SAC 2204 RT Alone				
2342	RT Alone	23x19x7	23x11x9	24.0
2343	RT Alone	11x10x6	12x11x5	23.5
2344	RT Alone	Ø	Ø	23.0
2345	RT Alone	Ø	Ø	29.5
2346	RT Alone	Ø	Ø	24.4
2347	RT Alone	19x17x11	11x17x11	21.3
2348	RT Alone	14x12x10	14x12x10	26.8
SAC 2349 RT Alone				
2350	RT Alone	9x8x3	7x8x3	24.4
2351	RT Alone	8x7x3	9x6x2	26.4
2352	RT Alone	10x9x3	8x8x1.5	26.6
2353	RT Alone	9x5x1.5	8x5x2	25.5
2687	RT Alone	10x7x4	11x7x5	24.0
2688	RT Alone	9x9x4	11x11x4	25.6
2689	RT Alone	14x13x6	15x13x9	24.1
2690	RT Alone	8x7x2	7x7x3	26.9
2679	10^7 3636RT	Ø	Ø	22.7
2680	10^7 3616RT	Ø	Ø	
2681	10^7 3636RT	Ø 8x7x3	6x6x2	15.0
2682	10^7 3616RT	12x16x4	12x6x5	21.5
2691	10^7 3636RT	4x4x1.5	3x3x1.5	13.2
2692	10^7 3616RT	10x6x3	8x6x2	27.7
2693	10^7 3636RT	8x7x3	8x7x1.5	23.1
2694	10^7 3616RT	11x9x4	11x4x4	25.9
2683	10^7 3616RT	6x4x1.5	3x3x1.5	28.3
2684	10^7 3636RT	Ø	Ø	24.8
2685	10^7 3616RT	Ø	Ø	25.1
2686	10^7 3616RT	5x5x2	4x4x2.5	20.8
2675	10^7 8996RT	7x5x1.5	4x5x2	27.1
2676	10^7 8996RT	10x9x4.5	8x8x4	19.1
2677	10^7 8996RT	7x6x2	5x4x1.5	22.0
2678	10^7 8996RT	8x8x3	8x8x3	28.0

Data Sheet

2671	107	8996RT	2x2x1.5	2x2x1.5	22.2
2672	107	8996RT	5x3x1.5	2x2x1.5	25.5
2673	107	8996RT	9x7x3	1x7x2	18.6
2674	107	8996RT	6x4x1.5	4x3x1.5	22.9
2695	107	8996RT	2x6x3	6x5x2	21.7
2696	107	8996RT	6x6x2	16x11x2	20.9
2697	107	8996RT	9x7x6	7x7x3	22.5
2698	107	8996RT	10x9x5	11x9x4	16.8
2663	899.6		16x11x5	17x13x6	21.2
SAC 2664	899.6				
SAC 2665	899.6				
SAC 2666	899.6				
2703	899.6		21x16x7	21x19x7	27.3
2704	899.6		22x16x6	23x15x5	24.5
2705	899.8		18x15x8		
2706	899.6		18x18x6	17x15x4	19.7
2711	899.6		15x13x8		
2712	899.6		13x10x8		
2713	899.6		11x11x10		
2714	899.6		14x10x10		
2707	3616		13x10x4	15x13x4	27.2
2708	3616		11x10x6	12x11x6	23.4
2709	3616		17x13x7	20x16x9	26.9
2710	3616		17x13x7	17x15x10	25.7
2719	3616		5x3x1.5	2x2x1.5	23.9
2720	3616		5x4x1.5	2x2x1.5	26.3
2721	3616		11x9x3	13x11x6	24.8
2722	3616		7x6x2	6x6x4	24.5

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2189	RT Alone	1.5 x 1.5 x 1	1.0 x 1.0 x 1	25.0
2190	RT Alone	Ø	Ø	24.3
2192	RT Alone	Ø	Ø	24.3
2201	RT Alone	Ø	Ø	24.7
2202	RT Alone	Ø	Ø	30.7
2203	RT Alone	1.5 x 1.5	1.5 x 1.5 x 1.5	24.5
SAC 2204	RT Alone	Ø	Ø	Ø
2342	RT Alone	22 x 19 x 9	20 x 15 x 8	23.5
2343	RT Alone	12 x 10 x 4	13 x 12 x 6	27.7
2344	RT Alone	Ø	Ø	21.5
2345	RT Alone	Ø	Ø	24.7
2346	RT Alone	Ø	Ø	24.5
2347	RT Alone	20 x 18 x 9	transverse	Ø
2348	RT Alone	15 x 11 x 6	15 x 12 x 6	20.1
SAC 2349	RT Alone	Ø	Ø	Ø
2350	RT Alone	8 x 7 x 3	8 x 6 x 4	27.5
2351	RT Alone	10 x 7 x 3	9 x 7 x 4	26.0
2352	RT Alone	10 x 9 x 2	8 x 8 x 2	28.7
2353	RT Alone	8 x 5 x 2	8 x 5 x 3	26.7
2687	RT Alone	11 x 8 x 5	9 x 8 x 5	25.1
2688	RT Alone	10 x 9 x 4	10 x 9 x 5	28.3
2689	RT Alone	12 x 12 x 9	15 x 12 x 12	24.3
2690	RT Alone	9 x 6 x 3	7 x 6 x 3	28.5
2679	10^7 3636RT	Ø	Ø	24.9
SAC 2680	10^7 3636RT	Ø	Ø	Ø
2681	10^7 3636RT	12 x 8 x 5	12 x 9 x 5	23.3
2682	10^7 3636RT	Ø	Ø	Ø
2691	10^7 3636RT	7 x 5 x 1.5	Ø	23.1
2692	10^7 3616RT	Ø	6 x 6 x 1.5	24.0
2693	10^7 3636RT	7 x 7 x 1.5	8 x 6 x 1.5	24.6
2694	10^7 3616RT	10 x 7 x 4	10 x 7 x 3	26.6
2683	10^7 3616RT	Ø	Ø	24.6
2684	10^7 3636RT	Ø	Ø	22.0
2685	10^7 3616RT	Ø	Ø	4
2686	10^7 3616RT	4 x 3 x 1.5	1.0 x 1.0 x 1	2
2675	10^7 8996RT	4 x 4 x 1.5	4 x 4 x 1.5	27.3
2676	10^7 8996RT	8 x 8 x 4	9 x 7 x 4	20.3
2677	10^7 8996RT	1.5 x 1.5 x 1.5	4 x 4 x 1.5	24.7
2678	10^7 8996RT	9 x 8 x 3	8 x 6 x 3	29.5

HISTO-PATHOLOGICAL

3d 6d 9d
 Control
 RT alone
 RT + 3616
 3616 alone

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Data Sheet

2671	10*7	8996RT	Ø	Ø	24.5
2672	10*7	8996RT	Ø 1x1x1	Ø 1x1x1	28.7
2673	10*7	8996RT	12x5x1.5	6x3x1.5	22.7
2674	10*7	8996RT	1.5x1.5x1.5	1.5x1.0x1.0	24.3
2695	10*7	8996RT	5x5x3	0x4x2	24.9
2696	10*7	8996RT	11x8x5	12x12x6	24.3
2697	10*7	8996RT	7x6x3	7x5x2.5	25.3
2698	10*7	8996RT	14x12x7	10x9x5	15.1
2663	899-6		17x15x8	20x16x8	22.6
SAC 2664	899-6				
SAC 2665	899-6				
SAC 2666	899-6				
2703	899-6		21x21x5	21x21x7	25.9
2704	899-6		20x18x5	20x16x6	26.9
SAC 2705	899-6				
2706	899-6				
2711	899-6		18x15x8	16x17x10	24.2
2712	899-6		15x12x8	15x13x8	26.7
2713	899-6		15x12x9	15x16x8	28.9
2714	899-6		15x15x9	15x15x7.5	24.4
2707	3616		19x17x11	23x20x14	27.5
2708	3616		14x10x10	16x14x10	25.2
* 2709	3616		24x23x11		
2710	3616		14x17x12	19x17x12	15.2
2719	3616		Ø	Ø	22.9
2720	3616		Ø 10x10x7	11x9x6	24.6
2721	3616		16x14x6	17x17x8	28.4
2722	3616		Ø	Ø	26.0

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2342	RT Alone	20 x 14 x 8	20 x 14 x 8	26.2
2343	RT Alone	14 x 10 x 7	12 x 11 x 7	22.1
2344	RT Alone	Ø	Ø	20.10
2345	RT Alone	Ø	Ø	25.2
2346	RT Alone	Ø	Ø	25.4
SAC 2347	RT Alone	20 x 14 x 8	20 x 14 x 8	
2348	RT Alone	14 x 14 x 6	10 x 14 x 6	21.6
SAC 2349	RT Alone	20 x 14 x 8	20 x 14 x 8	
2350	RT Alone	10 x 8 x 4	10 x 8 x 4	28.1
2351	RT Alone	9 x 6 x 2	8 x 7 x 3	26.2
2352	RT Alone	11 x 7 x 2	9 x 7 x 3	29.7
2353	RT Alone	7 x 6 x 2	7 x 5 x 2	26.1
2687	RT Alone	9 x 7 x 4	10 x 7 x 4	24.7
2688	RT Alone	10 x 7 x 4	10 x 10 x 4	29.0
2689	RT Alone	13 x 11 x 3	13 x 12 x 5	23.6
2690	RT Alone	8 x 6 x 3	8 x 6 x 3	27.9
2679	10^7 3636RT	Ø	Ø	25.2
SAC 2680	10^7 3616RT	20 x 14 x 8	20 x 14 x 8	
2681	10^7 3636RT	12 x 10 x 6	12 x 10 x 9	22.6
SAC 2682	10^7 3616RT	20 x 14 x 8	20 x 14 x 8	
2691	10^7 3636RT	Ø	Ø	23.3
2692	10^7 3616RT	6 x 6 x 1.5	6 x 5 x 1.5	30.3
2693	10^7 3636RT	6 x 5 x 1.5	5 x 4 x 1.5	28.2
2694	10^7 3616RT	8 x 6 x 2	8 x 6 x 2	26.8
2683	10^7 3616RT	Ø	Ø	24.6
2684	10^7 3636RT	Ø	Ø	27.4
2685	10^7 3616RT	Ø	Ø	26.4
2686	10^7 3616RT	1.0 x 1.0 x 1.0	Ø	28.4
2675	10^7 8996RT	4 x 4 x 1.5	4 x 4 x 1.5	27.8
2676	10^7 8996RT	10 x 8 x 4	8 x 9 x 6	21.6
2677	10^7 8996RT	4 x 3 x 1.5	6 x 4 x 1.5	23
2678	10^7 8996RT	8 x 6 x 3	7 x 6 x 2	28.6
2671	10^7 8996RT	Ø	Ø	24.4
2672	10^7 8996RT	Ø	Ø	27.8
2673	10^7 8996RT	5 x 5 x 1.5	4 x 4 x 1.5	26.0
2674	10^7 8996RT	Ø	Ø	24.5
2695	10^7 8996RT	3 x 3 x 1.0	2 x 2 x 1.5	24.5
2696	10^7 8996RT	11 x 10 x 6	11 x 10 x 4	23.0
2697	10^7 8996RT	7 x 5 x 2	10 x 4 x 2	26.0

Data Sheet

	2698	1057 8996RT	10x7x6	10x7x5	16.5
	SAC 2683	899-6	21x19x13	21x19x13	
	SAC 2684	899-6	21x19x13	21x19x13	
	SAC 2685	899-6	21x19x13	21x19x13	
	SAC 2686	899-6	21x19x13	21x19x13	
	SAC 2703	899-6	26x23x11	26x23x11	
	SAC 2704	899-6	20x16x7	20x18x11	
	SAC 2705	899-6	20x16x7	20x18x11	
	SAC 2706	899-6	20x16x7	20x18x11	
	SAC 2711	899-6	22x20x7		
	SAC 2712	899-6	10x13x10		
	SAC 2713	899-6	21x22x12		
	SAC 2714	899-6	19x15x8	17x11x14	25.7
	SAC 2707	3616	21x19x13	21x19x13	
DCA	SAC 2708	3616	12x12x13		
	SAC 2709	3616	21x19x13	21x19x13	
DCA	SAC 2710	3616	17x15x11		
	SAC 2719	3616	Ø	Ø	23.8
	SAC 2720	3616	Ø	Ø	25.9
	SAC 2721	3616	22x20x9		
	SAC 2722	3616	13x12x6	14x12x6	24.6

	pre-ty	
2731	9x8x7	
2732 316 + RTX + GANE	2732	
2733	13x12x9	
2734		
2715	15x15x12	
2716	20x17x7	
2717	11x10x6	
2718	18x14x10	

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2346	RT Alone	ϕ	ϕ 2 25.3 ϕ
SAG 2347	RT Alone	-	-
2348	RT Alone	16x14x7	17x14x8 21.7
SAG 2349	RT Alone	-	-
2350	RT Alone	9x7x3	8x6x3 22.3
2351	RT Alone	8x6x3	8x7x3 25.8
2362	RT Alone	7x7x2	10x8x3 28.1
2363	RT Alone	7x5x3	7x5x2 26.3
2687	RT Alone	7x8x3	10x8x3 25.8
2688	RT Alone	8x11x10x4	11x9x4 28.9
2689	RT Alone	15x12x4	16x13x5 27.8
2690	RT Alone	8x6x3	7x5x3 28.2
2679	10^7 3636RT	ϕ	ϕ 25.3
SAG 2680	10^7 3616RT	-	-
2681	10^7 3636RT	13x12x9	13x12x8 21.0
SAG 2682	10^7 3616RT	-	-
2691	10^7 3636RT	6x5x1.5 \downarrow	6x4x1.5 29.2 \downarrow
2692	10^7 3616RT	ϕ	ϕ 22.1 \downarrow
2693	10^7 3636RT	4x4x1.5	4x3x1.5 24.7
2694	10^7 3616RT	6x5x2	6x6x2 27.0
2683	10^7 3616RT	ϕ	ϕ 24.0
2684	10^7 3636RT	ϕ	ϕ 23.3
2685	10^7 3616RT	ϕ	ϕ 26.2
2686	10^7 3616RT	1x1x1	ϕ 22.1
2675	10^7 8996RT	6x6x2	5x5x2 27.5
2676	10^7 8996RT	9x9x4	10x9x5 21.4
2677	10^7 8996RT	5x3x1.5	5x3x1.5 24.2
2678	10^7 8996RT	6x5x2	6x5x1.5 29.1
2671	10^7 8996RT	ϕ	ϕ 23.8
2672	10^7 8996RT	ϕ	ϕ 27.6
2673	10^7 8996RT	4x2x1.5	4x1.5x1.5 26.2
2674	10^7 8996RT	ϕ	ϕ 24.5
2695	10^7 8996RT	1x1x1	1x1x1 28.5
2696	10^7 8996RT	11x10x6	10x9x5 23.0
2697	10^7 8996RT	7x5x2	8x5x3 25.3
2698	10^7 8996RT	11x8x5	12x10x4 17.1
dead	2714 899-6	13x16x13	-

Data Sheet

2719	3616	ϕ	ϕ	23.9
2720	3616	6x3x1.5	6x4x2	27.2
SAC 2721	3616	15x15x6		
2722	3616	15x15x6	15x15x6	24.9
2715	HSV/GAN	18x15x11	25x15x10	deal 8/23
2716	HSV/GAN	21x18x10	23x18x15	21x17x11 21.8
2717	HSV/GAN	14x11x8	16x13x9	25.9
2718	HSV/GAN	22x18x10	23x18x15	23.6
2731	HSV/RT/GAN	6x6x1.5	6x6x3	23.4
2733	HSV/RT/GAN	13x13x4	12x11x3	23.6

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2346	RT Alone	Ø	
SAC 2347	RT Alone	Ø	
2348	RT Alone	16x14x7	
SAC 2349	RT Alone	Ø	
2350	RT Alone	7x6x3	
2351	RT Alone	7x7x3	
2352	RT Alone	8x7x5	
2353	RT Alone	7x4x3	
2687	RT Alone	16x8x4	
2688	RT Alone	9x6x4	
2689	RT Alone	16x15x8	
2690	RT Alone	6x6x2	
2679	10^7 3636RT	Ø	
SAC 2680	10^7 3616RT	Ø	
2681	10^7 3636RT	13x11x7	
SAC 2682	10^7 3616RT	Ø	
2691	10^7 3636RT	Ø	
2692	10^7 3616RT	4x3x1.5	
2693	10^7 3636RT	1x1x0.5	
2694	10^7 3616RT	3x3x1.5	
2683	10^7 3616RT	Ø	
2684	10^7 3636RT	Ø	
2685	10^7 3616RT	Ø	
2686	10^7 3616RT	Ø	
2675	10^7 8996RT	4x3x2	
2676	10^7 8996RT	8x8x3	
2677	10^7 8996RT	4x3x1.5	
2678	10^7 8996RT	1x1x0.5	
2671	10^7 8996RT	Ø	
2672	10^7 8996RT	Ø	
2673	10^7 8996RT	1x1x0.5	
2674	10^7 8996RT	Ø	
2695	10^7 8996RT	Ø	
2696	10^7 8996RT	7x8x3	
2697	10^7 8996RT	6x6x3	
2698	10^7 8996RT	12x10x5	
SAC	2714 899-6	Ø	

Data Sheet

2719	3616	Ø	
2720	3616	6x6x3	
SAC 2721	3616	6x6x3	
2722	3616	16x15x6	
AC 2723	HSV/GAN		
2716	HSV/GAN	18x16x8	
2717	HSV/GAN	18x13x7	
2718	HSV/GAN		
2735	HSV/GAN	14x10x6	
2731	HSV/RT/GAN	5x4x3	
2733	HSV/RT/GAN	16x10x8	

④ 2732 injected 9x9x6

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2687	RT Alone	11x9x3	25.5
2688	RT Alone	8x8x3	29.3
2689	RT Alone	19x17x8	27.8
2690	RT Alone	8x6x3	28.5
2679	10^7 3636RT	Ø	15.6
SAC 2680	10^7 3616RT	13x13x7	20.7
2681	10^7 3636RT	13x13x7	20.7
SAC 2682	10^7 3616RT	13x13x7	20.7
2691	10^7 3636RT	Ø	23.5
2692	10^7 3616RT	Ø	30.2
2693	10^7 3636RT	Ø	15.1
2694	10^7 3616RT	9x9x2	25.8
2683	10^7 3616RT	Ø	25.5
2684	10^7 3636RT	Ø	23.7
2685	10^7 3616RT	Ø	26.7
2686	10^7 3616RT	Ø	21.8
2675	10^7 8996RT	5x4x3	21.5
2676	10^7 8996RT	8x6x3	23.7
2677	10^7 8996RT	5x3x1.5	21.4
2678	10^7 8996RT	ØxØxØ.5	30.4
2671	10^7 8996RT	Ø	24.8
2672	10^7 8996RT	Ø	21.2
2673	10^7 8996RT	1x1x1.5	22.8
2674	10^7 8996RT	Ø	25.6
2695	10^7 8996RT	1x1x1	24.7
2696	10^7 8996RT	8x8x3	23.7
2697	10^7 8996RT	11x9x5	16.2
2698	10^7 8996RT	13x11x6	20.0
2719	3616	Ø	24.3
2720	3616	7x7x3	28.4
SAC 2721	3616	17x15x6	26.4
2722	3616	17x15x6	26.4
SAC 2715	HSV/ GAN	20x16x8	24.9
2716	HSV/ GAN	20x16x8	24.9
2717	HSV/ GAN	4x18x10	26.7
SAC 2718	HSV/ GAN	24x20x13	24.7

9x7x3
7x6x3
20x18x6
3x5x2

Ø
—
13x13x8

Ø
Ø
Ø
9x7x2

Ø
Ø
Ø
Ø

4x4x3
3x6x4
4x2x1.5
1x1x0.5

Ø
Ø
Ø
Ø

4x4x1
1x6x2
13x9x6
13x13x5

Ø
10x9x6

20x18x8

20x16x8
24x20x13

Data Sheet

ingent

2735	HSV/GAN		
2731	HSV/RT/GAN	5x3x2	23.5
2733	HSV/RT/GAN	1x9x0.5	24.8
2732	HSV/RT/GAN		

1x13x10

5x3x1.5
6x6x0.5
11x10x8

ingent

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2687	RT Alone	9x6x3	26.1	7x5x2
2688	RT Alone	8x5x2	28.8	6x6x4
2689	RT Alone	19x16x4	24.5	13x14x6
2690	RT Alone	8x6x4	29.7	6x5x2
2679	10^7 3636RT	0	25.8	0
SAC 2680	10^7 3616RT	0	0	mm
2681	10^7 3636RT	15x13x8	20.7	15x14x8
SAC 2682	10^7 3616RT	0	0	mm
2691	10^7 3636RT	0	24.2	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.0	0
2694	10^7 3616RT	7x7x2	25.0	10x8x4
2683	10^7 3616RT	0	25.8	0
2684	10^7 3636RT	0	23.8	0
2685	10^7 3616RT	0	22.2	0
2686	10^7 3616RT	0	22.2	0
2675	10^7 8996RT	5x4x3	29.7	4x3x2
2676	10^7 8996RT	6x6x4	24.8	5x5x5
2677	10^7 8996RT	0.5x0.5x0.5	25.7	0.5x0.5x0.5
2678	10^7 8996RT	1x1x0.5	30.5	0.5x0.5x0.5
2671	10^7 8996RT	0	24.8	0
2672	10^7 8996RT	0	28.3	0
2673	10^7 8996RT	0	27.6	0
2674	10^7 8996RT	0	25.5	0
2695	10^7 8996RT	7x5x2	26.2	8x6x3
2696	10^7 8996RT	9x6x3	23.4	5x3x1.5
2697	10^7 8996RT	13x11x6	27.0	15x10x7
2698	10^7 8996RT	15x15x5	20.0	15x15x6
2719	3616	0	25.3	0
2720	3616	10x10x2	27.3	11x10x6
SAC 2721	3616	0	0	mm
SAC 2722	3616	19x21x10	—	mm
SAC 2715	HSV/ GAN	0	0	mm
2716	HSV/ GAN	21x18x8	22.9	23x18x10
SAC 2717	HSV/ GAN	0	0	mm
2718	HSV/ GAN	0	0	mm
2735		15x12x8	29.7	16x11x6

Data Sheet

2730	HSV-QAN		
2731	HSV/RT/GAN	372 X 1.5	25.6
2732	HSV/RT/GAN	525 X 1.5	25.8
2732	HSV/RT/GAN	13 X 10 X 8	22.0

1X2735
0
12X8X6

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2687	RT Alone	18.5 x 2	26.4	6x5x1.5
2688	RT Alone	18.2 x 2	24.6	8 20x18x8
2689	RT Alone	6x6x4	30.2	5x5x4
2690	RT Alone	6x4x2	28.1	6x4x2
2679	10^7 3616RT	0	26.3	0
SAC 2680	10^7 3616RT	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
2681	10^7 8996RT	16.0 x 6	21.3	14x13x7
SAC 2682	10^7 3616RT	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
2691	10^7 3616RT	0	25.1	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3616RT	0	26.8	0
2694	10^7 3616RT	10x9x6	24.7	12x10x6
2683	10^7 3616RT	0	26.1	0
2684	10^7 3616RT	0	24.0	0
2685	10^7 3616RT	0	27.0	0
2686	10^7 3616RT	0	22.4	0
2675	10^7 8996RT	5x4x3	29.3	5x5x1
2676	10^7 8996RT	0	25.4	0
2677	10^7 8996RT	0.5x0.5x0.5	25.3	0.5x0.5x0.5
2678	10^7 8996RT	0.5x0.5x0.5	30.1	0
2671	10^7 8996RT	0	25.2	0
2672	10^7 8996RT	0	28.8	0
2673	10^7 8996RT	0	28.0	0
2674	10^7 8996RT	0	26.0	0
2695	10^7 8996RT	1x0x3	25.2	7x5x2
2696	10^7 8996RT	0	23.0	0
2697	10^7 8996RT	14x10x6	21.3	14x10x6
2698	10^7 8996RT	15x13x7	19	16x14x4
2719	3616	0	25.1	0
2720	3616	13x12x5	28.4	14x11 5
SAC 2721	3616	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
SAC 2722	3616	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
2715	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
2716	HSV/ GAN	12x10x5	26.7	12x6x4
2717	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX
2718	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX

Data Sheet

SAC	2735	HSV/GAN	24.214	24.3	
	2731	HSV/RT/GAN	0.552.521.5	26	0
	2733	HSV/RT/GAN	0	23.7	0
	2732	HSV/RT/GAN	11.44.4	23.2	10.44.5

Sibery @ radonc.duke.edu

100

EFFICIENCY LINE 22.203

[illegible]

U-91 culture medium

Now start U-91 culture
for 10 days in vitro

Frozen cell line (U-91)

Media: prepare culture medium (U-91) (U-91)

500 cc DMEM

3/2/85

5% FCS (Fetal Calf Serum)

5% penicillin

5% streptomycin

See MEMA

Methods:

Place frozen cell aliquot into warm H₂O bath

Transfer cells to 5 ml of media in 10 ml flask drop by drop

Spin in centrifuge for 5' at 200-300 rpm

Aspirate off supernatant & section

Resuspend cells in 2 ml of media (drop by drop)

Add 2 ml of cells to 3 ml in T-25 (drop by drop)

Place cells in incubator (top slightly loosened)

C-6 (C-6M) in neck mice

Radial Dose from H.J. to C-6 (P) in hind legs

	ear (g)	① hour	② hour	
5501	0			front limb
5502	1			② hr
5503	2			front limb
5504	1.0			② hr

infect mice to 10ml 3616 per altm

radiation of C-6 node

only 2 mice could be radiated due to human size
radiated for 20 Gy (10'28")
irradiated ① and ② LR

Harvest C-6 node into human in 2 mice (day 1)

	wt (g)	①	②	wt (g)	①	②
will harvest	5501	11x9x6mm	12x7x7	black	5.11g	5.16g
5501	5502	12x7x7		turnabout	5.64	5.33
5502				human	0.53g	0.17g
5503	5502	19x15x11mm	19x15x11mm	Black	5.13g	5.14g
				human	7.19g	7.12g
					2.02g	1.98g

Day 3 (9/26/5)

day 5 ← 5503
due to human size

Day 9 (9/28/5)

55004

21x16x15

23x20x16

22x16x15

17x14x14

5.15	5.21
6.6	9.6
7.1	2.39
5.6	5.12
5.10	5.01
8.03	6.60

D-54 (LPH) SR made mice (4/10/68)

Rec'd mice from H.O. of D-54 in (3) hind limb

early

(1) hind limb

(2) hind limb

5005

1

3x6x6 0

14x12 (30)

5006

2

11x10x6 (34)

12x10x6 (34)

5007

3

4.5x10x7 (34)

12x10x6 (34)

Inject mice a 10ul 3616 (2x10⁵ PFU) peritoneum

Injection of D-54 mice

inject 3 mice a 20ul to (2) hind limb

Harvest of D-54 mice tumors

Harvest 5007 on day 1 (10/5/68), 5005+5006 on day 2 (10/10/68)

5007

(1) mm³

14x9x8

(2) mm³

16x9x9

wt (g)

(1)

5.16

(2)

5.03

tumor

5.93

5.51

tumor

0.77

0.48

5005

16x10x8

11x6x5

wt

5.8

5.22

tumor

6.30

5.54

tumor

1.12

0.32

5006

22x15x12

16x12x9

wt

5.18

5.05

tumor

6.98

6.31

tumor

1.80

1.26

1. Vito D-54 G-6000

need

0 PFU (0.1% of 10⁶)

1 PFU (0.1% of 10⁶)

10 PFU (0.1% of 10⁶)

need 9 plates plus 1 plate
set up 12 plates

1) Trypsinize cells

2) Count cells

$$127 \times 12 \times 10^4 = 144 \times 10^5 = 144 \times 10^6$$

resuspend in 4 ml media

3) Add 1 ml media/plate and 1 ml cells soln

Infect plates 4-9 c. HSP-1 (1 PFU or 10 PFU)

~90% confluent

1) Count cells in 1 plate

$$30 \times 10^4 \times 10^4 = 3 \times 10^9$$

for 1 PFU want 3×10^6 PFU/plate

for 10 PFU " 3×10^7 PFU/plate

$$[36167] = 9 \times 10^9 \text{ PFU/ml}$$

$$\frac{3 \times 10^6 \text{ PFU/plate}}{9 \times 10^9 \text{ PFU/ml}} = \frac{1 \text{ ml}}{3 \times 10^3} \left(\frac{1000 \text{ ml}}{1 \text{ ml}} \right)$$

$$= \frac{1}{3} \text{ ml/plate}$$

3 PFU, not 10 PFU

need 3 plates

set up 9

$$\frac{3 \times 10^7 \text{ PFU/plate}}{9 \times 10^9 \text{ PFU/ml}} = \frac{1 \text{ ml}}{3 \times 10^2} \left(\frac{10^3 \text{ ml}}{1 \text{ ml}} \right)$$

$$= \frac{1}{3} \text{ ml/plate}$$

$$= 3.33 \text{ ml/plate}$$

$$= 13.33 \text{ ml in 12 media}$$

Add Virus 13.33 ml in 12 media

set up 4

EFFICIENCY LINE 22203

In vitro U-23 Cytokine

need 0.2 PFU $\left\{ \begin{array}{l} 0.6 \mu\text{g} \\ 2 \mu\text{g} \end{array} \right\} \begin{array}{l} 6^\circ \\ 24^\circ \end{array}$
 0.1 PFU $\left\{ \begin{array}{l} 0.3 \mu\text{g} \\ 1 \mu\text{g} \end{array} \right\} \begin{array}{l} 6^\circ \\ 24^\circ \end{array}$
 10 PFU $\left\{ \begin{array}{l} 0.3 \mu\text{g} \\ 1 \mu\text{g} \end{array} \right\} \begin{array}{l} 6^\circ \\ 24^\circ \end{array}$

need 12 plates plus 1 for count cells
 i.e. 0 plates \Rightarrow by to set 12 plates

- 1) Trypsinise cells
- 2) Count cells $60 \times 24 \times 10^4 = 14.4 \times 10^6$ in 10^6 ml
- 3) Try to plate 2×10^6 cells/plate
- 4) Add 95% cell vol to 5% media/plate

Infect plates with HSV-1 (1 PFU or 10 PFU)
 ~80% confluent

- 1) Count cells in 20% plate $4.4 \times 10^4 \sim 50$
 $50 \times 10 \times 10^4 = 5 \times 10^6$ cells/plate

Infect 20 PFU (5×10^6 PFU) + 10 PFU (5×10^3 PFU)

7.3.9 $10^6 \times 10^3 \text{ ml/ml}$ 1 PFU $5 \times 10^6 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^7 \text{ PFU}} \right) = \frac{2.5}{10} \text{ ml} = 0.25 \text{ ml}$
 2 ml in 12 ml $\Rightarrow 250 \text{ ml/plate}$
 in 3 ml media 750λ in 12 ml

10 PFU $5 \times 10^3 \text{ PFU} \left(\frac{1 \text{ ml}}{9 \times 10^9} \right) = \frac{1}{2} \times \frac{1}{100} \text{ ml} \times \frac{100 \text{ ml}}{1 \text{ ml}} = 5 \text{ ml}$
 in 3 ml \Rightarrow make 12 ml add 20 ml virus

4.5.6 0.1 PFU $5 \times 10^5 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^9 \text{ PFU}} \right) = \frac{2.5}{100} \text{ ml} = 0.025 \text{ ml}$
 25 λ /p
 25 λ in 9 ml

In V. h. 0-251

med

0.1 PFU (10⁴)

0.1 PFU (10⁴)

10¹⁰ PFU (10⁴)

med 9 plates plus 1 plate for control
for up 12 plates

17
28
103

1) Prep

2) Count cells

3) Add 10ml media/plate + 1ml cell suspension

$$148 \times 12 \times 10^4 = 168 \times 10^4 = 16 \times 10^6 \text{ bacteria in 12ml}$$

Plates 80-90% confluent

Count cells in 12ml plate 28-30

$$80 \times 12 \times 10^4 = 960 \times 10^4 = 9.6 \times 10^6 = 9.6 \times 10^6$$

will infect 0.1 PFU and 1 PFU

0.1 PFU (10⁴)
PFU

$$10 \times 10^4 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^4 \text{ PFU}} \right) = \frac{5}{100} \times 10000 = 50 \text{ ml}$$

1 PFU (10⁶)
PFU

$$10 \times 10^6 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^6 \text{ PFU}} \right) = \frac{11}{100} \times 10000 = 11 \text{ ml/plk}$$

Wet at 94th cm

2) Harvest 4.5 L at 94th cm

3) Harvest plates

4) Plates 3, 6, 9, 24, 2980 (current total 60000)

1, 2, 4, 5

7, 8, 9, 6th

20, 20, 10, 10, 10

In Viko D-541 Glucose study (cont) from 10/6/45

After 29 (18%) additional 7% seen culture note
will include 110

Cells look round after 1x E-HSD at 1148 hr

96g for plate 23, 36, 39 at 1200
plan to harvest plates in 60 and 240 post tx
with 2"54" D, 250 kV per 6g

Harvest

Harvest plates 1, 2, 4, 5, 7, 8 at 60 post RT (600 pm)
and harvest remaining 3 plates at 240 (1200 10/10/45)

Harvest plates

* 3, 6, 9 at 240 post RT (1200)
6 97% confluent rounding
3 36% confluent rounding

EFFICIENCY INDEX 22 208

In vitro 1-82 Cytoblast (cont)

Reinforced 0.6 L-10 PFA at 130° am
2° post 1° add 2nd norm media

11. 11. 11

Incubate at 700 am (1-20°)

harvest at 200 pm (60 post 2T) add 300 am

152 4.8 2.8
Smaller all roundly
Spherical

harvest at 200 330 am

3
Same

9
lots of all roundly
defn

D-391 (GBM) in nude mice cytolysis

Area 3 mice from MIT - D-391 x (1) and log

early

(1) mice

(2) mice

SD03

0

14x9x6

(3) 10

10x10x12

(4) 10

SD04

1

10x11x8

(3) 10

15x10x12

(4) 10

SD10

1

14x7x5

(3) 10

13x7x10

(4) 10

injected mice - total 346 (216 SD03) per hour

Partial Mice

Injected 3 mice - 20 Gy to (1) and (2) mice
 3 SD03 partially irradiated 21 to lower size

Area Mice

Day 1

SD03

L_{mm^3}

F

$L(g)$

F

14x10x9

11x10x9

5.13

5.15

5.56

5.62

0.43

.47g

Day 3

SD03

17x15x12

18x15x14

5.17

5.11

6.73

7.06

1.56

1.98

SD10

12x10x8

11x9x6

5.10

5.03

5.73

5.39

0.70

.36

July
(4)



1/1 (2%)

2% (2%)



W / Dates

	1	2	3	4	5	6	7	8	9
1	Received from Joany Chou (7/21/94)								
2	• SK-N-SH human neuroblastoma cell cultures								
3	medium = 10% FCS in DME								
4	• R3616 (HSV 1 (8/23/95)) titer = 6×10^9 PFU/ml								
5	• R 899-6 (R3616 + TNF) titer ~ 6×10^9 PFU/ml (filtered in Re: 8/5/94)								
6									
7	Gave Joany Chou (7/21/94)								
8	• AT cells & medium								
9	• SCC 61 cells & medium								
10									
11	Vero cells (medium = 5% FCS in DME) received from								
12	Elena 7/28/94.								
13	199V media (virus) received 500 cc. Order from Gibco								
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Current medium for propagation: Eagle's MEM with non-essential amino acids, sodium pyruvate, 1 mM and Earle's BSS, 90%; fetal bovine serum, 10%.

This is one of a number of cell lines derived from malignant gliomas (See also ATCC HTB 16, 17) by J. Ponten and associates from 1966-69 (Acta Pathol. Microbiol. Scand. 74: 465-486, 1968; Hum. Hered. 21: 238, 1971). Cultures were established as explants on grid-supported lens paper or gelatin foam with Eagle's minimum essential medium and 10% bovine calf serum as the culture fluid. Trypsinization of the outgrowth or cells attached to the vessel floor with subsequent transfer to standard vessels in growth medium permitted cell line development. A culture at passage 108 was deposited by J. Ponten in July, 1973. Mycoplasma contamination was eliminated in September, 1975.

HUMAN TUMOR CELL BANK — HTB

ATCC HTB 14 (continued)

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-44; Sex-Female; Race-Caucasian; Blood Type-A⁺.

Grown as: Monolayer; transferred 1:5 weekly.

Morphology: Epithelial-like.

In Vitro Cytopathology: (P120) Consistent with glioblastoma.

Nude mouse: Produces malignant tumor consistent with glioblastoma.

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 122.

Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.

Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	2	3	1	15	21	7	1
Chromosomes:	40	41	42	43	44	45	47

The stemline chromosome number is hypodiploid, the 2S component occurring at 5.4%. Nine markers [t(1q;?3), t(1p22q), t(6p?11q-), t(6q?7p), t(7q;?), del(12q), t(20;1p;9q), t(78p;?), and M1] were common to most S metaphases. Neither HSR's nor DM's were detected. The line was originated from a female patient. However, all S metaphases were monosomic for the X chromosome.

Viability: 93%.

Culture Medium: Eagle's minimum essential medium with non-essential amino acids, sodium pyruvate and Earle's BSS, 85%; fetal bovine serum, 15%; antibiotic-free.

Isoenzymes: Me-2, 1; PGM₁, 1; PGM₁, 2; ES D, 1; AK1, 1; GLO-1, 1; G6PD, B.

Phenotype Frequency Product: 0.0017.

Sterility: Tests for mycoplasma, bacteria and fungi were negative.

Species: Confirmed as human by isoenzyme analysis.

Note: This material is available under the conditions that you will not use it for commercial purposes or distribute it to third parties. Please see pages xv and xvi for the form required. Price Code: J

ATCC HTB 15

U-118 MG

(Glioblastoma, human)

Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%.

This line is one of a series derived by J. Ponten and associates as discussed under ATCC HTB 14, 16 and 17 (Acta Pathol. Microbiol. Scand. 74: 465-486, 1968). The source tumor was described as a grade III astrocytoma-glioblastoma with one area resembling an ependymoblastoma. Cytoplasmic granulation was striking and astroblasts with neurofibrils were observed. Spongioblasts were abundant in culture and were not affected by frequent subcultivation.

A culture at passage 416 was provided originally by J. Ponten. Progeny transferred to the ATCC in 1982 were found to be contaminated with mycoplasma. The infection was cured in 1987 by treatment with BM cycline over a 6-week culture period.

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-50; Sex-Male; Race-Caucasian; Blood Type-A⁺.

Grown As: Monolayer; transferred 1:3.5 weekly.

Morphology: Mixed.

Karyology: Hypopentaploid to hyperpentaploid with abnormalities including breaks (P419).

In Vitro Cytopathology: All spindle giant cells, malignant.

Nude mouse: Yields pleomorphic malignant tumor consistent with glioblastoma multiforme invading muscle.

HLA Cell Line Phenotype: AW 24, 28; B12, W47 (Pollack, et al.).

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 443.

Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.

Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	1	1	3	1	2	4	1	4	1	3	4	2	3	3	5	1	2	2	2	1	1	2	1
Chromosomes:	71	99	102	103	104	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	123	125

CELL REPOSITORY LINES — CRL

ATCC CRL 1690

†Passage Frozen: 427. Current medium for propagation: Eagle's MEM with non-essential amino acids, 1.0 mM sodium pyruvate and Earle's BSS, 90%; fetal bovine serum, 10%. Additional Information: This line was derived by L. Hayflick from a glioblastoma multiforma tumor from a 61-year-old male Caucasian. T98G has an indefinite lifespan and is anchorage-independent, but can enter a viable G1-arrested state when crowded or deprived of serum. These cells should be useful for studies on the mechanisms for cessation of proliferation in quiescent cells, and for studies requiring cells synchronized in G1 phase. This is a hyperpentaploid human cell line. The modal chromosome number should be around 128 to 132. The rate of cells with higher ploidies was 1.39%. Fourteen to 16 marker chromosomes were common to most cells. Reference: J. Cell. Physiol. 99: 43-54, 1979. Submitted by: G.H. Stein, University of Colorado, Boulder, CO. Price Code: J

ATCC CRL 1691

C7 (Mouse hybridoma, anti LDL receptors)

Complete description appears in the Hybridoma Section of the Catalogue (pp. 333-343).

ATCC CRL 1692

HISM (Smooth muscle, jejunum, human)

†Passage Frozen: 12; PDL 16. Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%. Additional Information: This cell line was derived from the *muscularis propria* of the jejunum of a normal 35-year-old female patient. It synthesizes collagen and contains actin stress fibers. It contracts in response to the C-terminal octapeptide of cholecystokinin. Reference: Proc. Soc. Exp. Biol. Med. 176: 503-507, 1984. Submitted by: M.F. Graham and R.F. Diegelmann, Medical College of Virginia, Richmond, VA. Price Code: J

ATCC CRL 1693

NFS-5 C-1 (Pre-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-2SM ecotropic murine leukemia virus. The cells bear a pattern of markers consistent with identification as large pre-B lymphoblasts (i.e., Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). The cells spontaneously produce both ecotropic and mink cell focus-forming viruses. Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1694

NFS-70 C-10 (Pro-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-NS-7 ecotropic murine leukemia virus. The cells bear a pattern of markers suggestive of pro-B lymphoblasts (i.e., Mac-1+, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). The cells appear to be of a very early stage of commitment to B-cell differentiation. Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1695

NFS-25 C-3 (Pre-B lymphoblast, mouse)

†Passage Frozen: Unknown. Current medium for propagation: Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 µg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. 129: 751-758, 1982). Additional Information: This line was derived from a spontaneously arising lymphoma in an NFS.C58v-1 mouse. The cells bear a pattern of markers consistent with identification as pre-B lymphoblasts (i.e., Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB-, sIg-, Ia-, and Ly-1+). Reference: J. Immunol. 133: 744-753, 1984. Submitted by: W.J. Davidson, and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1696

McCoy (Mouse)

†Passage Frozen: Unknown. Current medium for propagation: Eagle's MEM with non-essential amino acids and Earle's BSS, 90%; fetal bovine serum, 10%. Additional Information: Little descriptive information about the origin of the McCoy cells appears in the literature. They were first mentioned by Pomerat, *et al.* (Z. Zellforsch. 47: 158-174, 1957). The cells have originated from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis. It was shown that McCoy cells (designated McCoy A) were indeed human cells. However, another subline was shown in fact, of mouse origin and possessed marker chromosomes characteristic of strain L mouse. Initial interest in McCoy cells followed the demonstration by Gordon and Quan (Proc. Natl. Acad. Sci. 65: 1065-1069, 1968) and Gordon, *et al.* (Appl. Microbiol. 23: 123-129, 1972) that ionizing radiation induced infection of McCoy cells to chlamydia strains. A culture of the so-called McCoy cells was made available to the National Disease Control, Cell Culture Department, Atlanta, GA in March, 1984. The cell line has been used to propagate laboratory strains of chlamydia. The cell line has been satisfactory for chlamydia growth for at least 10 years. Price Code: J

(Receptors)

(pp. 333-349).

sample				TUF pg/ml			
SR	MOI	XR	extract	SR	MOI	XR	cell
HS	5	24	24h	SR20B	MOI 5	24h	54
HS	"	"	"	"	"	"	26
SR	MOI 0.1	XR 24	"	"	"	"	63
HS	"	"	"	"	"	"	54
SR	MOI 5	XR 6h	"	SR20B	MOI 0.1	"	24h
HS	"	"	"	"	"	"	62
SR	0.1	"	"	"	"	"	200
HS	"	"	"	"	"	"	134
SR	5	"	"	"	"	"	16
HS	"	"	"	"	"	"	7
SR	0.1	"	"	"	"	"	33
HS	"	"	"	"	"	"	11
SR	5	"	"	"	"	"	14
HS	"	"	"	"	"	"	3
SR	0.1	"	"	"	"	"	257
HS	"	"	"	"	"	"	477
SR	5	"	"	"	"	"	480
HS	"	"	"	"	"	"	60
SR	0.1	"	"	"	"	"	240
HS	"	"	"	"	"	"	255
SR	5	"	"	"	"	"	240
HS	"	"	"	"	"	"	194
SR	0.1	"	"	"	"	"	278
HS	"	"	"	"	"	"	429

median of

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16

X .2 ml = pg / 10^7 cells

24 is best

SR killed

HS not ?

titrate down virus, time (< 24h), & serum

7/27

Decanted CM

confluent x 4 days unfed

$\frac{1}{500}$ MSV \pm XR (36g) 6 hrs later

18hr decant & feed

80% or 20% of each onto confluent cells

all $\frac{1}{500}$ MSV

Ø fed

Ø fed \rightarrow subculture

36g fed

36g fed \rightarrow subculture

20% Ø CM

20% Ø CM \rightarrow subculture 24°

20% 36g

20% 36g \rightarrow subculture 24°

80% Ø

80% Ø \rightarrow sub 24°

80% 36g

80% 36g \rightarrow sub 24°

24h CM Ø \rightarrow ? sub 24° ? \pm XR

24h CM 36g \rightarrow ? sub 24° ? \pm XR

48h CM Ø

48h CM 36g

$$\begin{array}{r} 0 \\ 40 \quad 122 \quad 200 \\ 100 \quad 200 \quad 500 \end{array}$$

$$\begin{array}{r} 0 \\ 90 \quad 157 \quad 200 \\ 500 \quad 10 \quad 10^4 \end{array}$$

$$\begin{array}{r} 0 \\ 79 \quad 100 \quad 236 \\ 200 \quad 100 \quad 500 \end{array}$$

$$\begin{array}{r} 0 \\ 100 \quad 250 \quad 71000 \\ 200 \quad 500 \quad 10 \quad 10^4 \end{array}$$

Confluent

$$\begin{array}{r} 61 \quad 43 \quad 162 \\ 200 \quad 100 \quad 500 \end{array}$$

Confluent

$$\begin{array}{r} 253 \quad 1000 \quad 500 \\ 500 \quad 10^4 \quad 10^3 \end{array}$$

$$\begin{array}{r} 0 \quad 0 \quad 0 \\ 200 \quad 10^3 \quad 500 \\ 0 \quad 0 \quad 0 \\ 200 \quad 10^3 \quad 500 \end{array}$$

cell cycle affected?

? toxin or
depletion or
virus or
artifact (over topping
to confluent)

8/2/43

1) ? does cell density effect PE 2) ? is a new protein made after treatment

40% PE
300, 500, 10^3 , 2×10^3 , 5×10^3 , 3×10^4 → 3 Gy
↓
4% "

} on dishes for 1 hr
in 1 ml medium

② SQ20B 10^5 in T-75 (2)

③ SQ20B 5×10^5 on p100 - 4 dishes → 35S-methionine

PE 3 Gy

40	$\frac{30}{300}$	$\frac{30}{500}$	$\frac{200}{2000}$	$\frac{25}{5000}$	$\frac{15}{10^4}$	$\frac{20}{300}$	$\frac{20}{500}$	$\frac{30}{1000}$	$\frac{10}{5000}$	$\frac{25}{10^4}$
		$\frac{100}{1000}$						$\frac{100}{2000}$		
4	$\frac{30}{300}$	$\frac{50}{500}$	$\frac{70}{5000}$	$\frac{20}{10^4}$		$\frac{10}{300}$	$\frac{25}{500}$	$\frac{0}{2000}$	$\frac{40}{5000}$	$\frac{40}{10^4}$
		$\frac{100}{1000}$	$\frac{120}{2000}$							

EFFICIENCY LINE 22-206

1 What is the relative infectivity of HSV 3616 in SQ-20B compared to Vero cells?

2 What level of ~~infectious~~ viral inoculum can mice tolerate?

3 At what PFU is TNF production maximized?

4 At what time point is TNF production maximized?

5 Compare wt HSV I to HSV 3616 to HSV 899-6

6 Does RT affect TNF production?

7 How does RT affect cytotoxicity?

where does HSV alone fall?

SURV

- KR
- (R899-6)
- TNF + KR
- (R3616)
- HSV + KR

8 Does supernatant sensitize? How about supernatant to Vero cells?

9 Does supernatant retain infectivity or toxicity?

10 Is TNF produced in viral infection with RT?

11

12

13

14

15

16

17

18

19

20

21

22

[illegible]

10 ml

990

EFFICIENCY LINE 22-206

[illegible]



MEDIUM 199/1% calf serum(pH7.6-7.9)

Application: virus propagation and titration.

Preparation: 1X 199v

room T° sterile glass distilled water.....440mls
10X 199 Hank's BSS w/o L-glutamine(Hazleton)..... 50mls
1000X Pn/Strept.....0.5mls
heat inactivated bovine calf serum(Colorado Serum).. 5mls
100X L-glutamine(Sigma)..... 5mls
7.5%(w/v)NaHCO₃(Sigma).....9.35mls

(1) NaHCO₃ must be added last.

(2) NaHCO₃ concentration must be 1.4g/l in the 1X formulation. The color of the 1X medium after addition of NaHCO₃ should be cherry red. If necessary, add 6N NaOH dropwise to achieve the desired color. DO NOT increase amount of NaHCO₃.

(3) Store at 4°C; shelf life 4-6 weeks.

Preparation: 1X 199v

1X 199v.....500mls
pooled human gamma globulin.....0.5mls

To 500mls 1X 199v aseptically add 0.5mls(0.1%) pooled human immunoglobulin.

Shelf life: suggest preparing fresh.

Gibco

Medium 199 = Cat No 11181-021

10 X

10 X Hank's salts

10 X L-glut

10 X NaHCO₃

GIEMSA STAIN

Application: Solution used to stain viable cells; specifically used in cell culture for virus titration assay.

Source: Sigma

Preparation: 10X GIEMSA

Time Element: 16 day period

giemsa powder.....5 grams

glycerol.....500mls

methanol.....500mls

- Day1
- (1) Put giemsa and glycerol into a flask which has a layer of glass beads .
 - (2) Place flask on a shaker at 37°C; shake overnight.

- Day2
- (3) Remove flask from 37°C and add methanol.
 - (4) Mix(with stir bar)at room temperature .
 - (5) Place in dark for 2 weeks(This can be achieved by completely wrapping flask with foil.) at room temperature.

- Day16
- (6) Filter solution with Whatman #1 paper.
 - (7) Store at room temperature. No known expiration.

Strengths:

Stock: 10X

Working: 1X (Prepare day of use by diluting 10X stock 1:10 with glass distilled water;
eg: 1ml 10X + 9mls water).

use 7.5ml (10 x stock) in ~~50 ml~~ + 42.5 ml dH₂O

SQ20B: 3616 vs 899-6, 10^{-1} vs 10^{-3} MOI

8/3/94-1

SIBLEY

EFFICIENCY LINE 22-206

Purpose

- Quantify cell killing in R3616 & R899-6 vs. no virus following 5 Gy RT.
- Does the TNF construct in R899-6 lead to additional cell kill over R3616?
- Quantify TNF production in serum and pellet after RT & infection.
- Compare cell killing with and TNF production with MOI of 10^{-1} , 10^{-3} .

Design

- Fixed Variables - Cell type: SQ-20B
RT dose: 5 Gy single fraction
Interval infect \rightarrow RT: 16 hrs
Interval RT \rightarrow subculture: 4 hrs
- Study Variables - Virus: None, R3616, R899-6
MOI: 10^{-1} PFU/cell, 10^{-3} PFU/cell

8/3/94 - 2

SIBLEY

Methods -

8/8/94 ① Plate 10^6 SQ-208 cells / 60 mm dish.

Volume/dish = 3-4 ml 20% FCS in DME + P/S

Incubate to 95% confluency (overnight)

Label Plates: 10^{-1} MOI R3616 10^{-3} MOI R3616(duplicate) 10^{-1} MOI R3616 10^{-3} MOI R3616 10^{-1} MOI R899-6 10^{-3} MOI R899-6 10^{-1} MOI R899-6 10^{-3} MOI R899-6

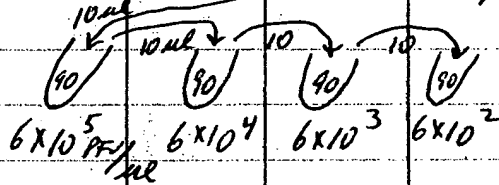
Virus

Virus \therefore need 10 plates +

1 extra to count cells

8/9/94 ② Count a plate using hemocytometer = 1.12×10^6 cell.

③ Make dilution of viruses:

R3616 = 6×10^9 PFU/ml = 6×10^6 PFU/ μ lR899-6 = 6×10^9 PFU/ml also, \therefore make same dilution.

④ Change media on plates to 2% FCS in DME, 2 ml/pl.

⑤ Add virus (12 noon)

For 10^{-1} MOI need $\frac{1.12 \times 10^6 \text{ PFU}}{6 \times 10^4 \text{ PFU}/\mu\text{l}} = 1.87 \mu\text{l}$ of 6×10^4 dilution.For 10^{-3} MOI need $\frac{1.12 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU}/\mu\text{l}} = 1.87 \mu\text{l}$ of 6×10^2 dilution.(optimally want vol of viral inoculum $\sim 2-3 \mu\text{l}$)

TNF-ELISA RESULTS

EFFICIENCY LINE 22-206

ELISA

Standards

0 med.	1	9	17
15.7	2	10	
33.1	3	11	18
62.5	4	12	
125	5	13	19
250	6	14	
500	7	15	20
1000	8	16	

Put TNF in 1ml distilled H_2O

1. dilute standards in 0.5ml R05 buffer (1:2)
2. Add standards (+ media only) 200ul to duplicate wells
3. Add 200ul of sample to each well, cover
shake 1 hr.
4. Wash 3X by aspirating liquid, adding ~200ul of wash buffer

5. Add 200ul of conjugate A
shake ~30 min.

6. Wash again
(make up A+B)

7. A 200ul A+B

1. 3616 10^{-3} cm

17. Ø v. ns cm

2. 3616 10^{-3} P

18. Ø v. ns cm

3. 3616 10^{-1} cm

19. Ø v. ns P

4. 3616 10^{-1} P

20. Ø v. ns P

5. 3616 10^{-1} P

9. 899-6 10^{-3} cm

10. 899-6 10^{-3} P

11. 899-6 10^{-1} cm

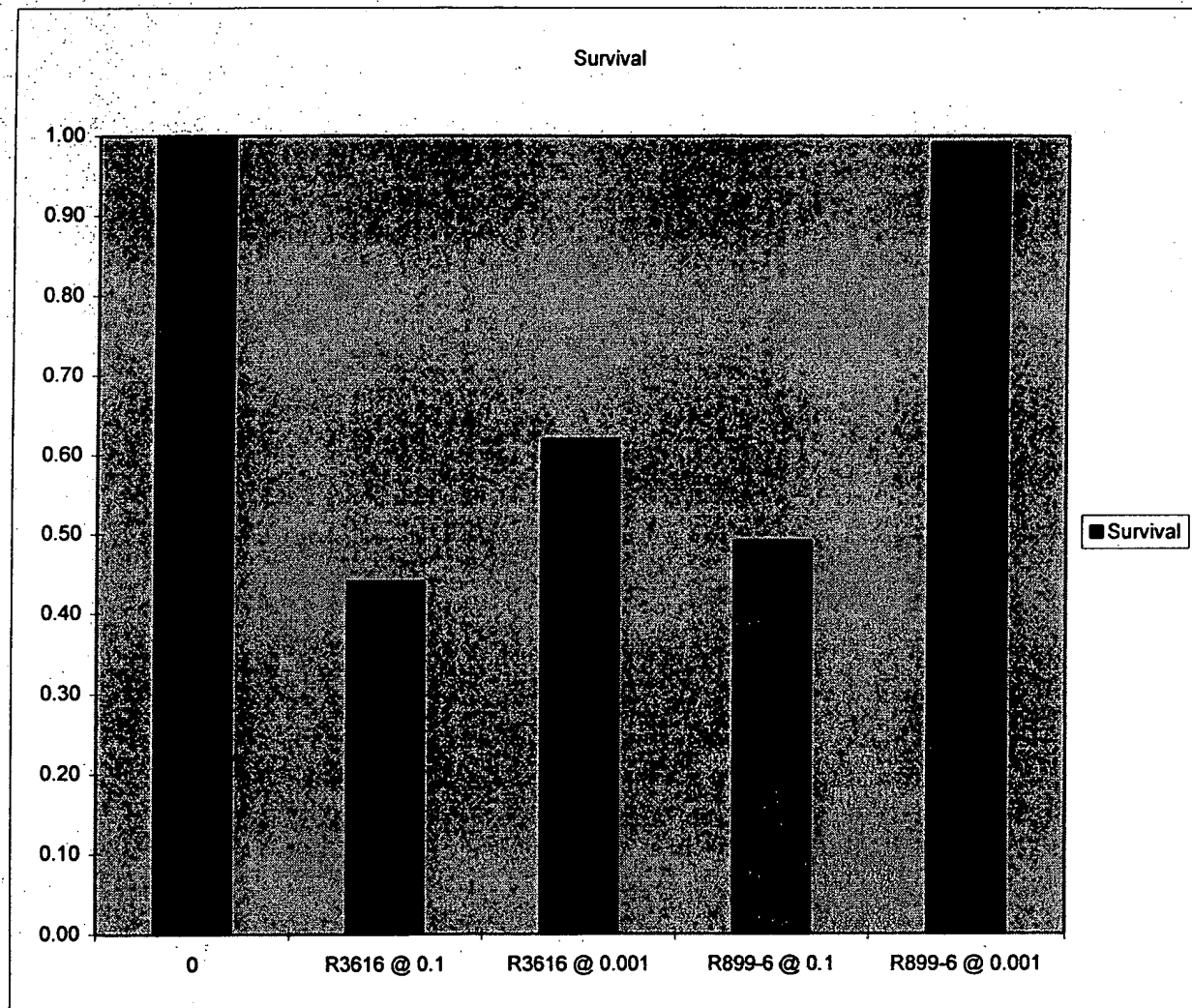
12. 899-6 10^{-1} P

13. 899-6 10^{-1} cm

14. 899-6 10^{-1} P

8/3/94 Results - Counts SQ20B					
RT	Virus	# Plated	Ave. Coun	P.E.	Survival
5	0	1000	176	0.176	1.00
5	R3616 @ 0.1	1000	77.5		0.44
5	R3616 @ 0.001	1000	109		0.62
5	R899-6 @ 0.1	1000	86.5		0.49
5	R899-6 @ 0.001	1000	174.5		0.99
Virus	Survival				
0	1.00				
R3616 @	0.44				
R3616 @	0.62				
R899-6 @	0.49				
R899-6 @	0.99				

SQ



8/3/94 - 6

	1	2	3	4	5	6	7	8	9	
1	<u>Findings</u>									
2	1.) In SQ-208:									
3	a. No detectable TNF production @ MOI 10^{-3} - 899-6 (+ 5 Gy)									
4	b. TNF detectable at MOI 10^{-1} (899-6 + 5 Gy) with 55 pg/ml in									
5	pellet sample & 32 pg/ml in supernatant sample.									
6	(pellet sample = 10^6 cells/ml)									
7	c. Despite TNF production, R899-6 did not produce more									
8	cell killing than R3616 (@ MOI 10^{-1} + 5 Gy)									
9	d. Both viruses caused increased cell killing at an									
10	MOI of 10^{-1}									
11	e. R3616 appeared to give more effective cell killing than									
12	R899-6 or Ø virus at MOI of 10^{-3} .									
13										
14										
15										
16										
17										
18										
19										
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21										
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25										
26										
27										
28										
29										
30										
31										





The University of Chicago Departmental Purchase Order

Purchase Order Number
Z 849078
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

American Type Culture Collection
12301 Parklawn Dr.

Rockville MD 20852
City State Zip Code

Payment Terms Delivery charge? ☐ Yes ☐ No

Telephone No. (800) 639-6597 FAX No.

THE UNIVERSITY OF CHICAGO

Dept. Code: 1050

Radiation and Cellular Oncology

Michael Beckett G-03
Ann. Room

5830 S. Ellis Ave.

Chicago IL 60637
City State Zip Code

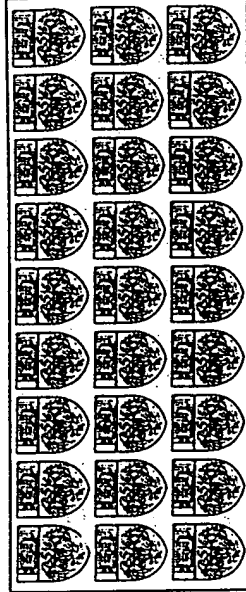
IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; second copy should be sent to vendor; third copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with department code number from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature Payroll No.
Muhammad Ghazvi 2-
Print Name Ext.
Account Code 5-25754-5400 Date 8/8/94

Order placed by phone? ☐ No ☒ Yes
Alonzo 8/8/94
Order placed with (name) Date
Michael Beckett 24833
Order placed by (name) Phone #



QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	Flask	ATCC HTB 14	75.00	75.00
		U-87 MG Cell Line	35.00	35.00
		Lab Fee	5.00	5.00
		Pkg. Fee	9.75	9.75
		Fed. Express		
		Acct. # 2168		

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

ORDER TOTAL

12075

MOLECULAR DEVICES

Vmax KINETICS MICROPLATE READER

PLATE #: 1

ASSAY

DATE: 8/11/94

OPERATOR: SIBLEY

O.D. LIMIT: 3.000

TIME: : AM/PM

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: OFF

NOTES:

CAL: ON

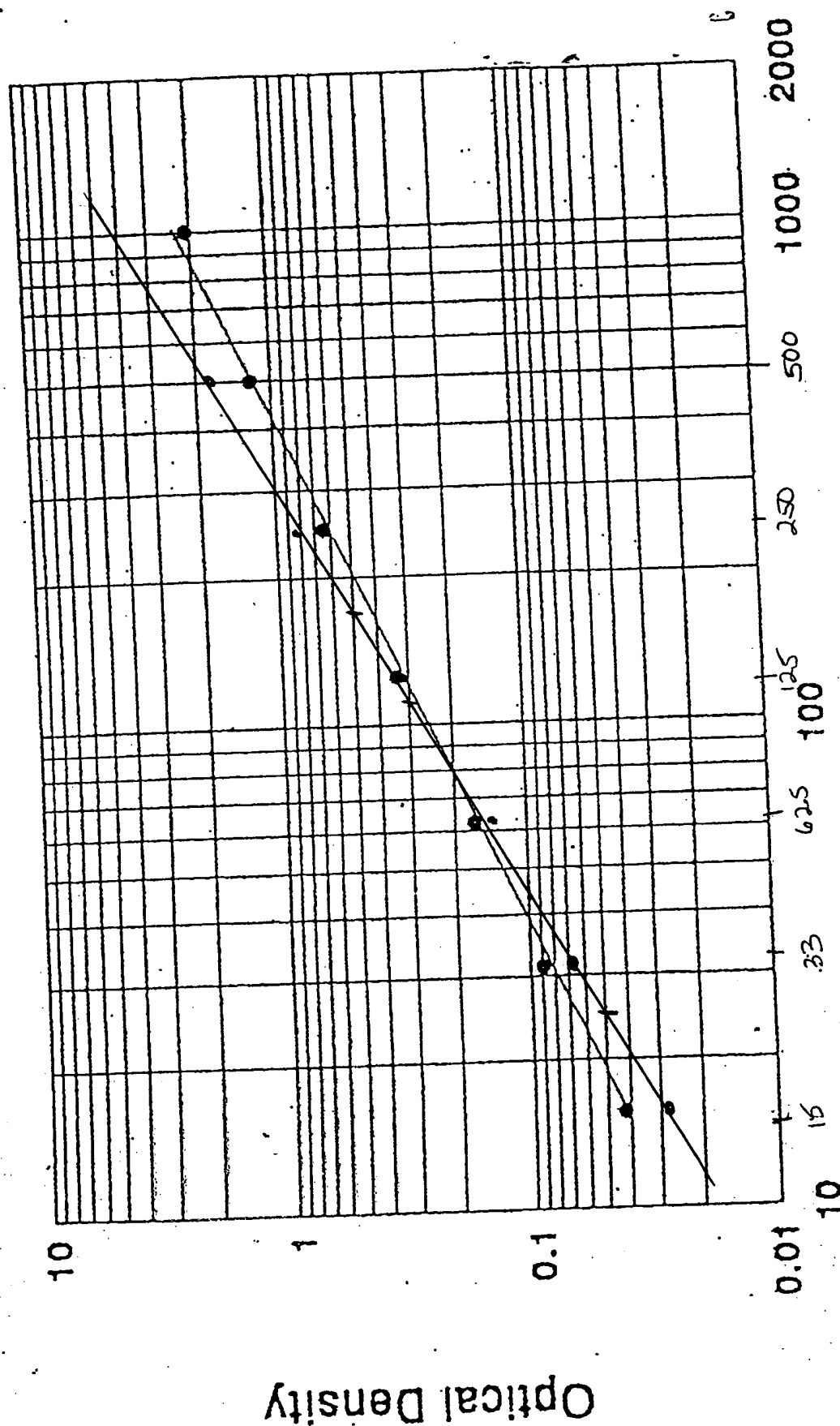
3616 499-6 0.051

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.052	0.054	0.049	0.051	0.046	0.047	0.047	0.000	0.000	0.000	0.000	0.000
B	0.030	0.078	0.045	0.056	0.045	0.048	0.046	0.000	0.000	0.000	0.000	0.000
C	0.121	0.120	0.045	0.044	0.047	0.047	0.051	0.000	0.000	0.000	0.000	0.000
D	0.213	0.207	0.044	0.051	0.047	0.078	0.039	0.000	0.000	0.000	0.000	0.000
E	0.363	0.332	0.048	0.048	0.106	0.099	0.081	0.000	0.000	0.000	0.000	0.000
F	0.927	0.896	0.046	0.047	0.048	0.101	0.047	0.000	0.000	0.000	0.000	0.000
G	1.823	1.803	0.047	0.046	0.045	0.172	0.050	0.000	0.000	0.000	0.000	0.000
H	*	*	0.049	0.050	0.185	0.197	0.050	0.000	0.000	0.000	0.000	0.000

Handwritten notes in table:
Row A: 0.028, 0.027
Row B: 0.069, 0.041
Row C: 0.161, 0.158
Row D: 0.311, 0.326
Row E: 0.878, 0.844
Row F: 1.777, 1.851
Row G: 1.814
Row H: 0.051
Row 5: 0.051, 0.049, 0.120, 0.139
Row 6: 0.051, 0.049, 0.120, 0.139
Row 7: 0.051, 0.049, 0.120, 0.139
Row 8: 0.051, 0.049, 0.120, 0.139
Row 9: 0.051, 0.049, 0.120, 0.139
Row 10: 0.051, 0.049, 0.120, 0.139
Row 11: 0.051, 0.049, 0.120, 0.139
Row 12: 0.051, 0.049, 0.120, 0.139

TNF- α Standard Curve

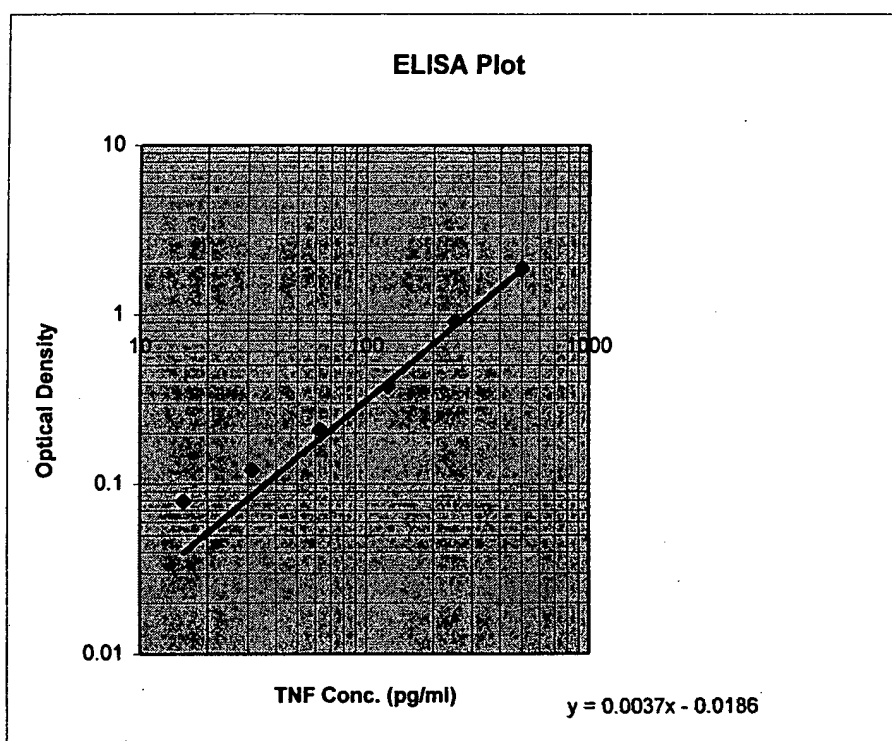
Diluent RD5



Concentration of TNF- α (pg/mL)

ELISA Plotting Worksheet

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
0.106	0.099	32.7	0.1025
0.101	0.101	32.3	0.101
0.172	0.172	51.5	0.172
0.185	0.197	56.6	0.191



T98G: 899-6 ~~only~~ @ MOI 10^{-1}
RT 0, 2, 5, 20 [TNF]

8/16/94 - 1

SIBLER

Purpose

- Quantify killing of T98G (glioblastoma cells) with R899-6 virus and 0, 2Gy, 5Gy & 20 Gy RT.
- Determine TNF production (? inducibility) with varying RT doses by ELISA.

Design

- Fixed Variables - Cell type: T98G

RT

Virus: R899-6 @ MOI of 10^{-1}

Interval infection → RT: 16 hrs

Interval RT → subculture: 6 hrs

- Study variables -

RT dose: 0, 2Gy, 5Gy, 20Gy

Aliquot media for TNF: ^{prior to RT} 30 min, 1 hr, 2 hr, 4 hr


Virus: None, R899-6

$4 \times 2 \times 2 (\text{dupl}) = 16 \text{ plates}$

8/16/94 -3

TNF ELISA RESULTS

(Note: error in standards, in old std curve used)

Cell type: T986 (Gibco/Star) Virus: R899-6 @ 10^6 cfu/ml

RT	Aliquot Time	Virus	[TNF]	Controls (No Virus)	
0 Gy	30'	899-6	N/A	0 Gy	
	1°		N/A	2 Gy	
	2°		49	5 Gy	
	4°		64	20 Gy	
	6°		94	} No TNF	
	Pellet (10^5 /ml)		23 = $230 @ 10^6$ /ml		
2 Gy	30'	899-6	59		
	1°		67		
	2°		(115)		
	4°		78		
	6°		73		
	Pellet (10^5 /ml)		25 = $250 @ 10^6$ /ml		
5 Gy	30'	899-6	46		
	1°		50		
	2°		54		
	4°		70		
	6°		101		
	Pellet (10^5 /ml)		24 = $240 @ 10^6$ /ml		
20 Gy	30'	899-6	56		
	1°		83		
	2°		66		
	4°		82		
	6°		127		
	Pellet (10^5 /ml)		27 = $270 @ 10^6$ /ml		

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
1° 0.181	0.181	53.9	0.181
0.143	0.143	43.7	0.143
2° 0.226	0.24	68.0	0.233
0.2	0.201	59.2	0.2005
4° 0.291	0.293	83.9	0.292
0.369	0.362	103.8	0.3655
6° 0.066	0.067	23.0	0.0665
0.069	0.063	22.9	0.066
0.243	0.253	72.1 30'	0.248
0.152	0.143	44.9	0.1475
0.157	0.167	48.8 1°	0.162
0.303	0.287	84.8	0.295
0.532	0.517	146.8 2	0.5245
0.175	0.173	52.1 6	0.174
0.314	0.336	92.9 6	0.325
0.203	0.195	58.8 4	0.199
0.345	0.342	97.9 4	0.3435
0.293	0.286	83.3 2	0.2895
0.062	0.067	22.5	0.0645
0.076	0.086	26.9	0.081

0.157	0.158	47.6	0.1575
0.142	0.146	<u>43.9</u>	0.144
0.148	0.151	45.4	0.1495
0.191	0.179	<u>55.0</u>	0.185
0.183	0.192	55.7	0.1875
0.177	0.17	<u>51.9</u>	0.1735
0.234	0.251	70.6	0.2425
0.231	0.238	<u>68.4</u>	0.2345
0.346	0.337	97.3	0.3415
0.371	0.36	<u>103.8</u>	0.3655
0.063	0.075	23.7	0.069
0.064	0.073	23.5	0.0685
0.182	0.203	57.1	0.1925
0.17	0.193	<u>54.1</u>	0.1815
0.208	0.247	66.5	0.2275
0.348	0.358	<u>100.4</u>	0.353
0.246	0.248	71.8	0.247
0.205	0.209	<u>61.0</u>	0.207
0.261	0.273	77.2	0.267
0.301	0.306	<u>87.1</u>	0.3035
0.457	0.448	127.3	0.4525
0.456	0.437	<u>125.7</u>	0.4465
0.083	0.079	26.9	0.081
0.085	0.079	27.2	0.082

EFFICIENCY LINE 22-206

[illegible]



MOLECULAR DEVICES

Vmax KINETICS MICROPLATE READER

PLATE #: 1

ASSAY : _____

DATE: ____/____/____

OPERATOR: _____ O.D. LIMIT: 3.000

TIME: ____:____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

AUTO MIX: OFF

NOTES: _____ CAL: ON

OPTICAL DENSITY

	13	14	15	16	5	6	7	8	9	10	11	12
-2°												
A	0.246	0.248	0.053	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.205	0.203	0.053	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
-4°												
C	0.261	0.273	0.059	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.301	0.306	0.058	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
-6°												
E	0.457	0.448	0.056	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.466	0.437	0.055	0.052	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P												
G	0.083	0.079	0.056	0.055	0.001	0.000	0.000	0.000	0.000	0.001	0.000	0.000
H	0.085	0.079	0.059	0.057	0.001	0.001	0.000	0.000	0.000	0.000	0.001	0.000

MOLECULAR DEVICES

Vmax KINETICS MICROPLATE READER

PLATE #: 2

ASSAY : _____

DATE: ____/____/____

OPERATOR: _____ O.D. LIMIT: 3.000

TIME: ____:____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

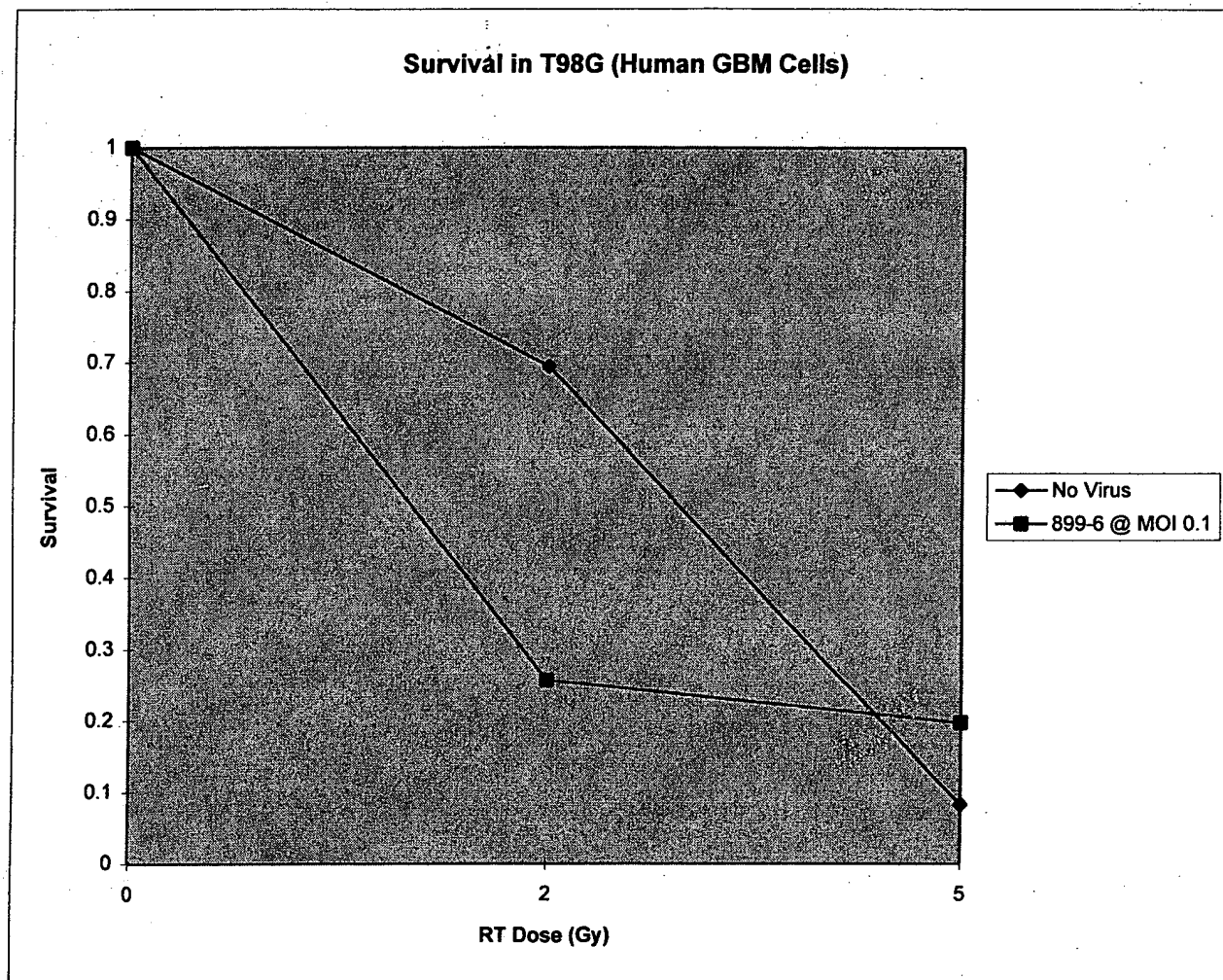
AUTO MIX: OFF

NOTES: _____ CAL: ON

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
			30'		6°				30'	56°	6°	
A	0.002	0.000	0.000	0.003	0.221	0.293	0.512	0.517	0.127	0.158	0.346	0.357
B	0.008	0.006	0.007	0.006	0.369	0.362	0.173	0.173	0.142	0.146	0.371	0.350
			1°		-P		6°		-1°		-P	
C	0.004	0.001	0.007	0.004	0.066	0.067	0.314	0.338	0.148	0.151	0.063	0.075
D	0.000	0.007	0.003	0.007	0.069	0.063	0.203	0.193	0.191	0.179	0.064	0.073
			2°		26°		4°		2°		30'	206°
E	0.002	0.006	0.061	0.181	0.243	0.253	0.345	0.342	0.183	0.192	0.182	0.203
			1°		2°		2°					
F	0.001	0.004	0.085	0.143	0.152	0.143	0.293	0.282	0.177	0.170	0.170	0.193

5/10



8/16/94 Results- Count Plates T98G Cells							
RT	Virus	# Plated	# Counted	#Counted	Average	P.E.	Survival
0	0	1000	154	104	0.129	0.129	1
		5000	tm	tm			
		10000	tm	tm			
2	0	1000	75	104	0.0895		0.693798
		5000	tm	tm			
		10000	tm	tm			
5	0	1000	18	13	0.0155		0.120155
		5000	20	44	0.0064		0.049612
		10000	71	124	0.00975		0.075581
0	899-6	1000	12	7	0.0095	0.015267	1
	(MOI=0.1)	5000	68	139	0.0207		
		10000	156	156	0.0156		
2	899-6	1000	3	6	0.0045		0.294118
		5000	13	17	0.003		0.196078
		10000	25	60	0.00425		0.277778
5	899-6	1000	4	2	0.003		0.196078
		5000	18	11	0.0029		0.189542
		10000	38	24	0.0031		0.202614
RT Dose	No Virus	899-6 @ MOI 0.1					
0	1	1					
2	0.694	0.256					
5	0.082	0.196					

8/16/94-4

	1	2	3	4	5	6	7	8	9	
1										
2	<u>RESULTS - Count plates (9/1/94)</u>									
3										
4	RT	Virus	# plated	# counted 1	# counted 2	counted ave.	P.E.	SURVIVAL	Ave SURVIVAL	
5	Ø	Ø	10^3	154	104	129	.13	100	100	
6			5×10^3	Tm	Tm	—	—	—		
7			10^4	Tm	Tm	—	—	—		
8	2 Gy	Ø	10^3	75	104	90	"	69	69	
9			5×10^3	Tm	Tm	—	—	—		
10			10^4	Tm	Tm	—	—	—		
11	5 Gy	Ø	10^3	18	13	16	"	12	8.1	
12			5×10^3	20	44	32	"	4.8		
13			10^4	71	124	98	"	7.5		
14						1.7	.017			
15	Ø	899-6	10^3	12	7	9.5	"	7.3	11.7	
16			5×10^3	68	139	103	"	15.9		
17			10^4	Tm	156	156	"	12		
18	2 Gy	899-6	10^3	3	6	4.5		3.5	4.3	.17
19			5×10^3	13	17	15	"	2.3		
20			10^4	25	(160)	25(2.5)	"	1.9(7.1)		
21	5 Gy	899-6	10^3	2	4	3	"	2.3	2.3	
22			5×10^3	18	11	14.5	"	2.2		
23			10^4	38	24	31	"	2.4		
24										
25										
26										
27										
28										
29										
30										
31										

8/16/94-5

FINDINGS -

- ① R899-6 virus is highly cytotoxic at an MOI of 10^{-1} to T986 cells with a plating efficiency of only 1.5%.
- ② Higher levels of TNF are produced in T986 cells than in SQ20B cells = 5 fold more at an MOI of 10^{-1} (250 vs 50).
- ③ No radiation induction of TNF, i.e. it is produced constitutively.
- ④ There is no clear synergistic effect between 5 Gy & R899-6 @ 10^{-1} , although there is possible synergy at 2 Gy.
- ⑤ T986 cells are more radiosensitive than SQ20B cells.

SQ 20B

8/26/94-1

EFFICIENCY LINE 22-206

Purpose-

- Quantify cell killing of R3616 at MOI of 10^{-2} , 10^{-1} , 5.
- Is killing additive or synergistic @ 5 Gy?
- Quantify the effect of IgG (human) in the incubation media
- ~~Does the IgG~~ Is there a cell density dependent killing, and does IgG eliminate this phenomenon

Design

- Fixed Variables - Cell type: SQ 20B
Virus: R3616
Interval from infection: 2° then
Interval from infection to RT: 16°
Interval from RT to subcult: 4-6°
- Study Variables - Virus: None, R3616 @ MOI 10^{-2} , 10^{-1} , 5
Human IgG: +, -
RT: 0 Gy, 5 Gy

8/26/44-2
SQ-20B

EFFICIENCY LINE 22-206

	1	2	3	4	5	6	7	8	9
1	Virus	moi	IgG	RT					
2	Ø	-	0	0	5.6				
3	"	"	"	"	4.8				
4	Ø	-	+	0	6				
5	Ø	-	0	5	3.4				
6	"	"	"	"	3.7				
7	Ø	-	+	5	4.2				
8	3616	10^{-2}	0	0	4.2				
9	"	"	"	"	inf.				
10	3616	10^{-2}	+	0	5.9				
11	"	"	"	"	4.2				
12	3616	10^{-2}	0	5	2.7				
13	"	"	"	"	2.4				
14	3616	10^{-2}	+	5	2.8				
15	"	"	"	"	4.2				
16	3616	10^{-1}	0	0	5.9				
17	"	"	"	"	5.8				
18	3616	10^{-1}	+	0	3.3				
19	"	"	"	"	3.35				
20	3616	10^{-1}	0	5	2.2				
21	"	"	"	"	1.9				
22	3616	10^{-1}	+	5	1.8				
23	"	"	"	"	1.8				
24	3616	5	0	0	1.8				
25	"	"	"	"	2.5				
26	3616	5	+	0					
27	"	"	"	"					
28	3616	5	0	5					
29	"	"	"	"					
30	3616	5	+	5					
31	"	"	"	"					

switch

need 31 plates \times 5 ml = 155 ml
 \therefore resuspend in 165 ml
 10^7 cells in 165 ml = 3×10^5 / 5 ml

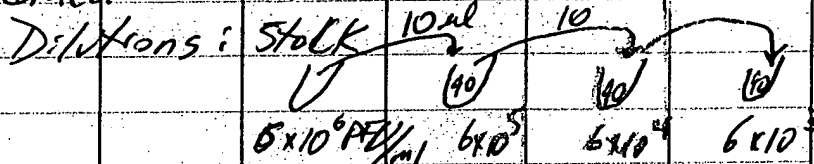
8/26/94 - 3

EFFICIENCY LINE 22-206

Methods -

8/26/94 ① Plate 5×10^5 cells per 60 mm dish

8/29/94 ② Infect \bar{c} virus in 2 ml 2% FCS



Count dish = 2×10^6 cells

- MOI 10^{-2} = $2 \times 10^4 \text{ PFU}$ / $6 \times 10^3 \text{ PFU/ml}$ = 3.3 ul of 6×10^3 dilution

- MOI 10^{-1} = $2 \times 10^5 \text{ cells}$ / $6 \times 10^4 \text{ PFU/ml}$ = 3.3 ul of 6×10^4 dilution

- MOI 5 = $1 \times 10^7 \text{ PFU}$ / $6 \times 10^5 \text{ PFU/ml}$ = 16.7 ul of 6×10^5 dilution

③ Incubate 2° then ~~add~~ aspirate media &

add: 4 ml of 2% FCS (\ominus IgG) or

4 ml of 2% FCS + human γ glob (1 ul/ml) (stock = $165 \text{ mg}/10 \text{ ml}$)

8/30/94 ④ Irradiate \bar{c} 0 or 5 Gy

⑤ Subculture & count each dish \bar{c} hemacytometer

⑥ Make dilutions 10^6 , 10^5 , 10^4 , 10^3 for each dish

⑦ Plate 3 dilutions for each original dish = 5×10^2 , 10^3 , 5×10^3

⑧ Incubate for 10 d - 14 d

[illegible]

SQ-203

	1	2	3	4	5	6	7	8	9	
1	VIRUS	MOI	IgG	RT	# PLATED	# COUNTED				
2	0	-	-	0	500	323				
3			-	0	500	203				
4			+	0	500	277				
5					$10^3/5 \times 10^3$	TM				
6	0	-	-	5	500	36				
7	"	"	"	"	"	72				
8	0	-	-	5	10^3	89				
9	"	"	"	"	"	130				
10	0	"	+	5	5×10^3	TM				
11	0	-	+	5	500	56				
12	"	-	+	5	10^3	140				
13	"	"	"	"	5×10^3	TM				
14	36/6	10^{-1}	-	5	500	51				
15	"	"	"	"	500	25				
16	"	"	+	"	500	31				
17	"	"	"	"	500	38				
18	"	"	+	"	10^3	68				
19	"	"	"	"	10^3	55				
20	"	"	+	"	10^3	59				
21	"	"	"	"	10^3	77				
22	"	"	-	"	5×10^3	215				
23	"	"	"	"	5×10^3	185				
24	"	"	+	"	5×10^3	246				
25	"	"	"	"	5×10^3	225				
26										
27										
28										
29										
30										
31										

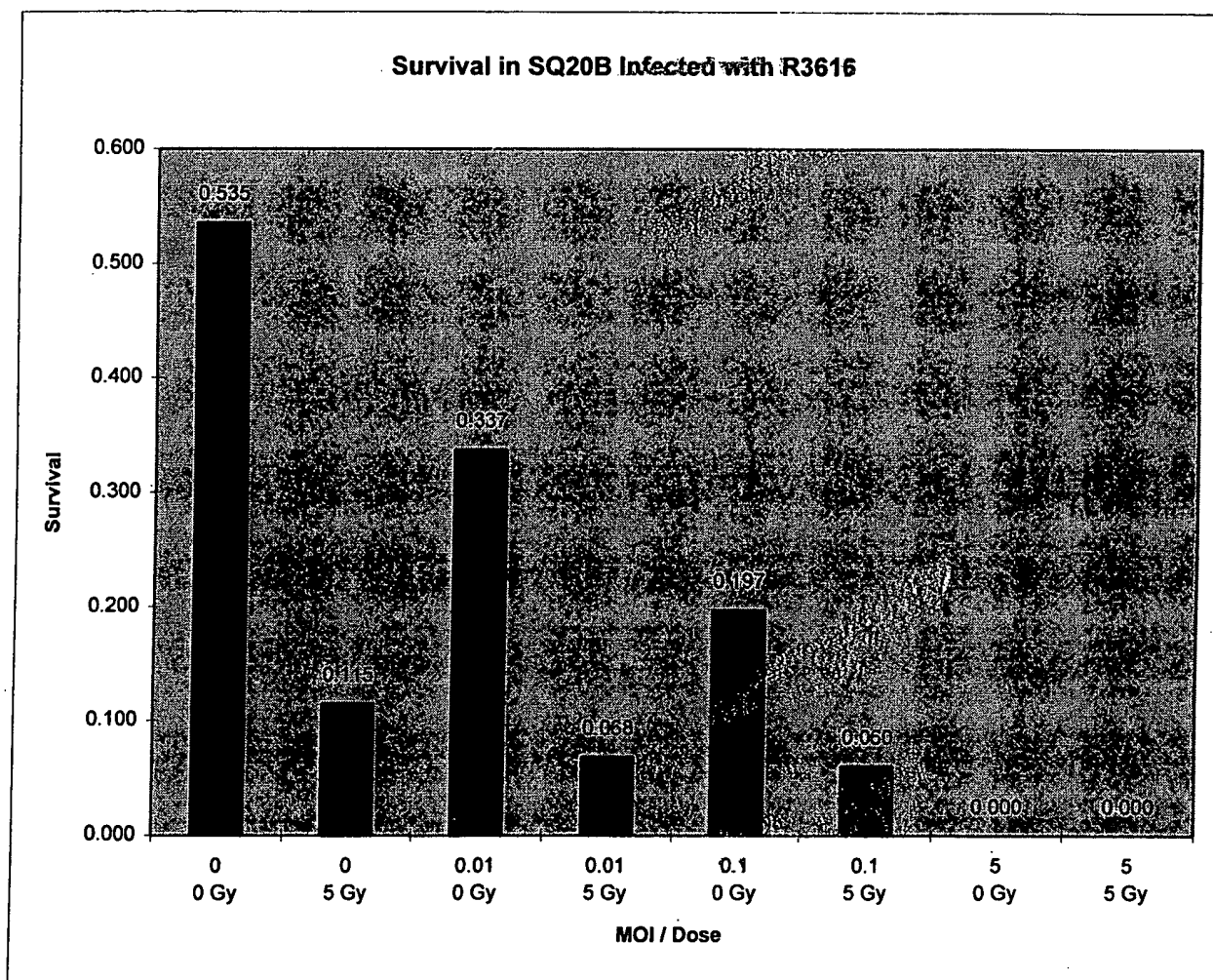
EFFICIENCY LINE 22-206



SQ 20B: R3616 BY MOI AND +/- IgG								
Virus	MOI	IgG	RT	# plated	# counte	Crude SV	PE	Rel. SV
0	-	-	0	500	323	0.646	0.535333	100
0	-	-	0	500	203	0.406		
0	-	+	0	500	277	0.554		
0	-	-	5	500	36	0.072	0.533	0.214822
0	-	-	5	500	72	0.144		
0	-	+	5	500	56	0.112		
0	-	+	5	1000	140	0.14		
0	-	-	5	1000	89	0.089		
0	-	-	5	1000	130	0.13		
R3616	0.01	-	0	500	214	0.428	0.3366	100
R3616	0.01	-	0	500	130	0.26		
R3616	0.01	+	0	500	177	0.354		
R3616	0.01	+	0	500	205	0.41		
R3616	0.01	-	0	1000	231	0.231		
R3616	0.01	-	5	500	49	0.098	0.337	0.202733
R3616	0.01	-	5	500	43	0.086		
R3616	0.01	+	5	500	25	0.05		
R3616	0.01	+	5	500	48	0.096		
R3616	0.01	-	5	1000	90	0.09		
R3616	0.01	-	5	1000	68	0.068		
R3616	0.01	+	5	1000	47	0.047		
R3616	0.01	+	5	1000	49	0.049		
R3616	0.01	+	5	5000	205	0.041		
R3616	0.01	+	5	5000	287	0.0574		
R3616	0.1	-	0	500	96	0.192	0.197	
R3616	0.1	+	0	500	101	0.202		
R3616	0.1	+	0	500	[251]	-		
R3616	0.1	-	0	1000	TM	-		
R3616	0.1	+	0	1000	197	0.197		
R3616	0.1	+	0	1000	TM	-		
R3616	0.1	-	5	500	51	0.102	0.197	0.305922
R3616	0.1	-	5	500	25	0.05		
R3616	0.1	+	5	500	31	0.062		
R3616	0.1	+	5	500	38	0.076		
R3616	0.1	-	5	1000	68	0.068		
R3616	0.1	-	5	1000	55	0.055		
R3616	0.1	+	5	1000	59	0.059		
R3616	0.1	+	5	1000	77	0.077		
R3616	0.1	-	5	5000	215	0.043		
R3616	0.1	-	5	5000	185	0.037		
R3616	0.1	+	5	5000	246	0.0492		
R3616	0.1	+	5	5000	225	0.045		
R3616	5	+	0	10000	0	0	0	0
R3616	5	+	5	10000	0	0		

SQ 20B: R3616 BY MOI AND +/- IgG

Dose	MOI	Survival
0 Gy	0	0.535
5 Gy	0	0.115
0 Gy	0.01	0.337
5 Gy	0.01	0.068
0 Gy	0.1	0.197
5 Gy	0.1	0.060
0 Gy	5	0.000
5 Gy	5	0.000



8/26/94

FINDINGS

In SQ-208:

- There is 63% & 37% survival @ MOI of 10^{-2} & 10^{-1} (corrected for plating efficiency)
- 5 Gy radiation results in 21% survival 5 virus (corrected for plating efficiency)
- Cell killing is additive between virus and radiation at the MOI & dose studied.
- An MOI of 5 results in complete cell killing
- Human γ -globulin in the ^{med} serum did not have an effect on survival
- No cell density dependence was observed

V 87 cells

8/29/94-1

EFFICIENCY LINE 22-206

Purpose -

- Compare cytotoxicity of R3616 & R899-6 in V-87 cells (human glioblastoma).
- Determine radiosensitivity of V-87 cells to 5 Gy.
- ~~Are~~ Is viral cytotoxicity additive or synergistic with radiation toxicity?
- Quantitate TNF production in R899-6 cells.
Does RT (5 Gy) increase TNF production?

Design -

- Fixed variables - cell type: V-87 (human glioblastoma)

Study variables -
Virus: None, R3616, R899-6
MOI: 10^{-3} , 10^{-1}
RT: 0, 5 Gy

V87 cells

8/29/44-3

EFFICIENCY LINE 22-206

Methods

① Plate 10^5 cells per 60 mm plate, Need 6 plate
9/3/44 ② When >90% confluent, infect w/ virus. Count cells in extra plate to determine PFU's.

Make dilutions of virus = 6×10^6 PFU/ml (stock) $\rightarrow 6 \times 10^5 \rightarrow 6 \times 10^4 \rightarrow 6 \times 10^3$

Count dish = 2.65×10^6

- mol $10^{-1} = \frac{2.65 \times 10^6 \text{ PFU}}{6 \times 10^4 \text{ PFU/ml}} = 4.4 \text{ ml}$

- mol $10^{-3} = \frac{2.65 \times 10^6 \text{ PFU}}{6 \times 10^2 \text{ PFU/ml}} = 4.4 \text{ ml}$

③ Incubate 20° in 20% FCS, then subculture dish & make dilutions = $10^6, 10^5, 10^4, 10^3$ cells/ml, 10^2

④ Plate dilutions:

Ø virus, Ø RT = 200, 500, 1000

Ø virus, 5 Gy = $5 \times 10^3, 10^4, 2 \times 10^4$

virus, Ø RT = $5 \times 10^2, 10^3, 5 \times 10^3, 10^4$

virus, 5 Gy = $5 \times 10^3, 10^4, 5 \times 10^4, 10^5$

Media = 10% FCS + P/S + 1% NaPy + 1% Ess a.a. in MEM $\pm 8966 \text{ lat/ml}$

⑤ Nat 40° , then irradiate

⑥ Incubate

⑦ Stain & Count

U-87

Note: poorly defined plaques, slow growing

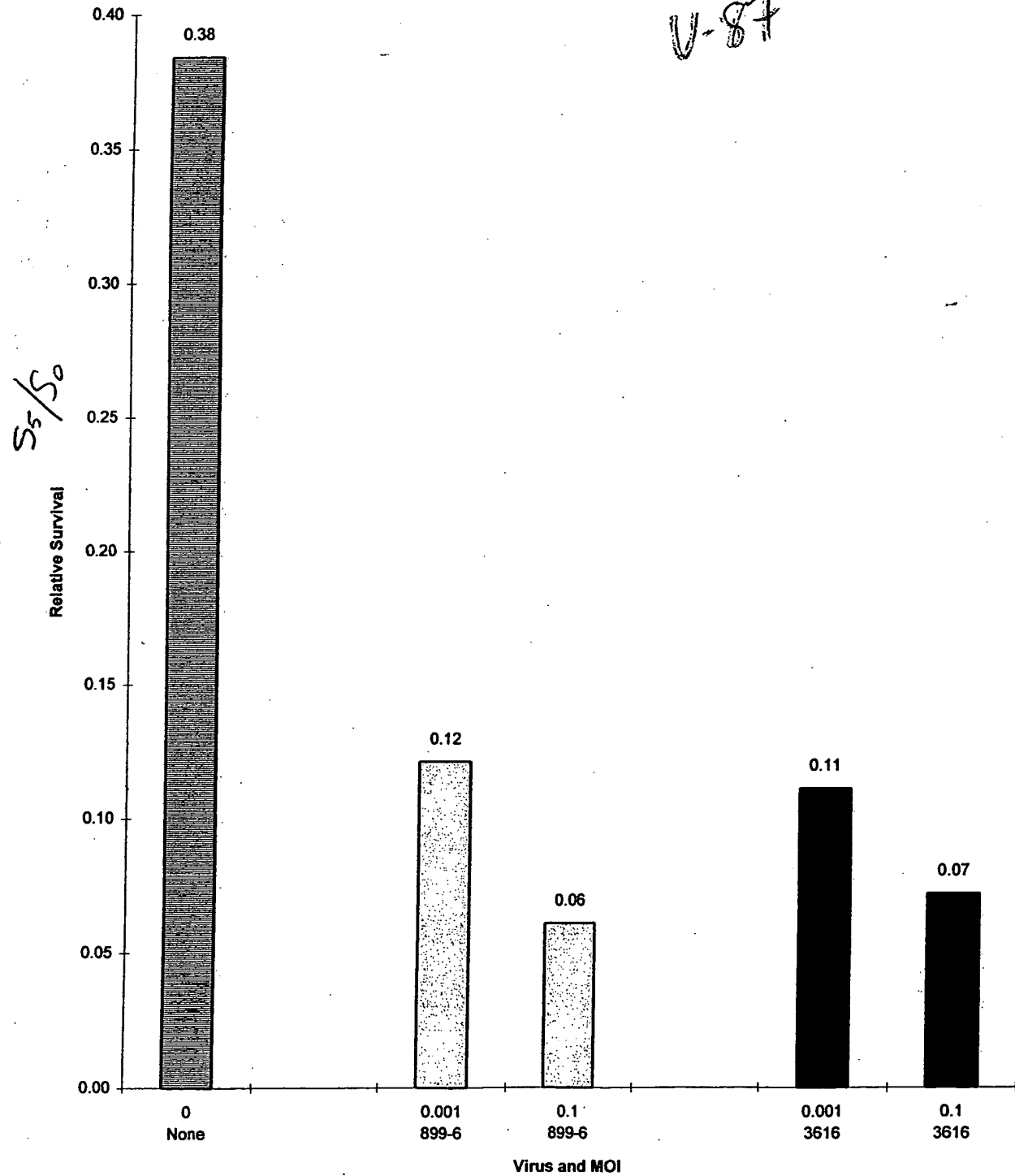
8/29/44 - 4

EFFICIENCY LINE # 22-206

AMRAD

	1	2	3	4	5	6	7	8	9
1	<u>Results -</u>								
2	vins	moi	RT	#plated	#counted	Conc SV	PE	Re/SV	
3	Ø	-	0	200	0/0				
4				500	0/1				
5				1000	6				
6	Ø	-	5	5000	1/2				
7				10 ⁴	0/12				
8				2x10 ⁴	42/45				
9	899-6	10 ⁻³	0	1000	21/6				
10				5000	61				
11				10 ⁴	?/1?				
12	899-6	10 ⁻³	5	5000	1/1				
13				10 ⁴	0/7				
14				5x10 ⁴	?/106				
15	899-6	10 ⁻¹	0	1000	35/6				
16				5000	125/69				
17				10 ⁴	?/1?				
18	899-6	10 ⁻¹	5	5000	1/0				
19				10 ⁴	6/8				
20				5x10 ⁴	138/148				
21	3616	10 ⁻³	0	1000	3/0				
22				5000	68/43				
23				10 ⁴	?/1?				
24	3616	10 ⁻³	5	5000	0/1				
25				10 ⁴	12/14				
26				5x10 ⁴	?/1?				
27	3616	10 ⁻¹	0	1000	14/8				
28				5000	106/?				
29				10 ⁴	?/1?				
30	3616	10 ⁻¹	5	5000	1/0				
31				10 ⁴	4/22				
				5x10 ⁴	?/104(?)				

Survival at 5 Gy Relative to Survival at 0 Gy



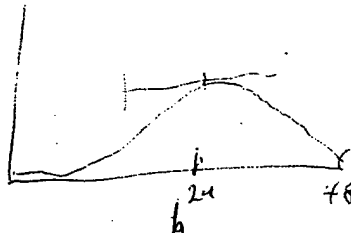
U-87 8/29/94									
RESULTS:									
Virus	MOI	RT	# plated	# counted		Crude SV	Ave SV	PE	Rel SV
None	-	0	200	0	0	0	0.002667	0.002667	1
None	-	0	500	1		0.002			
None	-	0	1000	6		0.006			
None	-	5	5000	1	2	0.0003	0.001025	0.002667	0.384375
None	-	5	10,000	0	12	0.0006			
None	-	5	20,000	42	45	0.002175			
899-6	0.001	0	1000	21	6	0.0135	0.00735	0.00735	1
899-6	0.001	0	5000	6		0.0012			
899-6	0.001	0	10,000	tm	tm				
899-6	0.001	5	5000	1	1	0.0002	0.00089	0.00735	0.121088
899-6	0.001	5	10,000	0	7	0.00035			
899-6	0.001	5	50,000	106	tm	0.00212			
899-6	0.1	0	1000	35	6	0.0205	0.01995	0.01995	1
899-6	0.1	0	5000	125	69	0.0194			
899-6	0.1	0	10,000	tm	tm				
899-6	0.1	5	5000	0	1	0.0001	0.00122	0.01995	0.061153
899-6	0.1	5	10,000	6	8	0.0007			
899-6	0.1	5	50,000	138	148	0.00286			
3616	0.001	0	1000	3	0	0.0015	0.0063	0.0063	1
3616	0.001	0	5000	68	43	0.0111			
3616	0.001	0	10,000	tm	tm				
3616	0.001	5	5000	0	1	0.0001	0.0007	0.0063	0.111111
3616	0.001	5	10,000	12	14	0.0013			
3616	0.001	5	50,000	tm	tm				
3616	0.1	0	1000	14	8	0.011	0.0161	0.0161	1
3616	0.1	0	5000	106	tm	0.0212			
3616	0.1	0	10,000	tm	tm				
3616	0.1	5	5000	1	0	0.0001	0.00116	0.0161	0.07205
3616	0.1	5	10,000	4	22	0.0013			
3616	0.1	5	50,000	104	tm	0.00208			

1/2

?

?

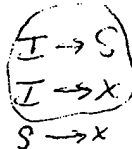
TNF



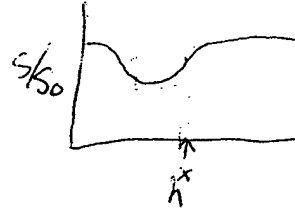
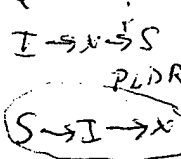
I -> ELISA

variable

time



Schedule



9/2/94-1

[illegible]

9/12/94

EFFICIENCY LINE 22-206

	1	2	3	4	5	6	7	8	9
1	TITERS								
2									
3	Cell	Virus	Dilution	Plaque #	Titer				
4	SQ 20B	3616	10^7	0	?				
5			"	0					
6			10^8	0					
7			"	0					
8	SQ 20B	899-6	10^7	0	?				
9			"	0					
10			10^8	0					
11			"	0					
12	V-87	3616	10^7	32 13	6×10^7				
13			"	8 8					
14			10^8	8 0					
15			"	7 0					
16	V-87	899-6	10^7	32 13	1.1×10^8				
17			"	8 8					
18			10^8	8 0					
19			"	7 0					
20	T-986	3616	10^7	14	1.2×10^8				
21			"	9					
22			10^8	0					
23			"	0					
24	T-986	899-6	10^7	12	4.1×10^8				
25			"	39					
26			10^8	3	8×10^8				
27			"	17					
28									
29									
30									
31									

none seen, but cells overgrown.
by filtering @ 80%
confluence

small
placques

nice
placques

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 18773

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASISREQUEST BY: Gary Sidley, MD DATE: 10/26/94REQUESTORS PHONE NUMBER: 312-702-0294AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 6-95150-5100 PI: HallahanVENDOR: Jackson Labs PROTOCOL: 58671REQUESTED DELIVERY DATE: ASAP PHONE: 2-6809SPECIES: mouse QUANTITY: 24STRAIN: nulj SEX: M F EITHERWEIGHT/AGE: 5-7 wks ALTERNATE WEIGHT/AGE: _____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

HOUSE AT: _____ CARLSON _____ WYLER _____ CLSC _____ FMI _____ OTHER _____

PROCUREMENT DESK: 2-9364

TITER ~~CELLS~~ VIRUSES in 4 cell lines

10/28/94

[illegible]

	1	2	3	4	5	6	7	8	9	
1	<u>VERO CELLS</u>									
2		D_1	D_2	D_3						
T-25	10^5	30%	40%	Sub conf 85%						
T-25	2×10^5	50%	80%	Conf -100%						
T-25	5×10^5	80-90%	-100%	Conf +						
T-150	10^5	-	-							
7										
8										
9										
10	<u>T986</u>	D_1	D_2	D_3						
T-25	5×10^4	-	30%							
T-25	10^5	-	50%							
T-25	2×10^5	30%	70%							
14										
15										
16	<u>V-87</u>									
17		D_1								
18	10^5									
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										

EFFICIENCY LINE 22/206



	1	2	3	4	5	6	7	8	9	
1	<u>In-vitro - SQ-20B</u>									
2	1.) Titer virus in SQ-20B									
3	2.) Generate Survival curve for R3616 @ 0, 3, 5, 7, 9, 11 Gy - MOI=0									
4										
5	<u>- U87</u>									
6	1.) Titer virus in U-87									
7	2.) Generate survival curve for R3616 (MOI = 10^{-3}) @ 0, 2, 3, 5, 7 Gy									
8	R3616 (MOI = 10^{-1})									
9	R899-6 (MOI = 10^{-3})									
10	R899-6 (MOI = 10^{-1})									
11										
12	<u>- T98G</u>									
13	1.) Titer virus in U-87									
14	2.) Generate survival curves as above for U87									
15										
16										
17										
18										
19										
20	<u>TNF</u>									
21	1.) Measure in U-87 & T98G infected w/ R3616 to									
22	R/p cellular TNF production in response to virus									
23	2.) Quantify									
24										
25										
26										
27										
28										
29										
30										
31										

EFFICIENCY LINE® 22-206

[illegible]

V-87

10/31/94-1

EFFICIENCY LINE® 22-206

PURPOSE -

- Establish dose response survival curve with V87 cell line
- Establish dose response survival curve with V87 cell line + R3616 @ $MOI = 10^{-1}$

DESIGN -

- Fixed variables - cell type V87 (human gbm)
- intervals = infect $\times 2^0 \rightarrow$ subcult $\rightarrow 4^0 \rightarrow$ XRT
- Study variables - virus: None, R3616 @ $MOI = 10^{-1}$
- dose: 0, 2, 3, 5, 7, 9 Gy

V-87

10/31/94-2

EFFICIENCY LINE 22-206

METHODS

① Plate V87 cells on 60 mm dish & grow to ~80% confluence. Need 5 plates. (plate 5×10^5 /plate)

11/2/94 ② Count 1 plate & infect 2 plates @ 10^1 10^{-1} (R3616) for 2°
 1.2×10^6 cells $\times 10^{-1}$ (moi) = 1.2×10^5 PFU
 $\frac{6 \times 10^5 \text{ PFU/ml dish}}{2 \text{ ml} \times .8 \text{ ml}}$

③ After 2° infection, subculture to 100mm plates (in duplicate)

RT	Virus	# plated		
0	-	500	1000	5000
2	-	1000	5000	10^4
3	-	5000	10^4	2×10^4
5	-	10^4	2×10^4	
7	-	10^4	5×10^4	10^5
9	-	5×10^4	10^5	
0	+	1000	2000	5000
2	+	5000	10^4	
3	+	10^4	2×10^4	
5	+	10^4	2×10^4	5×10^4
7	+	5×10^4	10^5	
9	+	5×10^4	10^5	

11/28/94 ④ Stain & count

10/31/94-3

[illegible]

U-87

10/31/44 - 4

	1	2	3	4	5	6	7	8	9
1	RESULTS (cont.)								
2									
3									
4	R361b mol	RT Dose	# Plated	# counted		ave Surv.	P.E.	rel Surv.	?
5	0.1	0	1000	0	0	3×10^{-4}	3×10^{-4}	1	
6			2000	0	0				
7			5000	2	1				
8		2	5000	0	0	3×10^{-4}		1	
9			10^4	3	3				
10		3	10^4	3	0	1.1×10^{-3}		3.7	
11			2×10^4	41	—				
12		5	10^4	2	0	1.4×10^{-3}		4.9	
13			2×10^4	20	23				
14			5×10^4	105	85				
15		7	5×10^4	36	16	8.7×10^{-4}		2.9	
16			10^5	92	116				
17		9	5×10^4	20	10	2.6×10^{-4}		.88	
18			10^5	4	45				
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



V-87

10/31/94-5

CONCLUSIONS

- ① V87 is a fairly radioresistant cell line
(surviving cells @ 9 Gy)
- ② Does not appear to be a major impact
of R3616 on dose response curve.
- ③ Plating efficiency extremely low ($= 5 \times 10^{-4}$)
Cannot draw any quantitative conclusions
- ④ Sit estimates SF₂ (in vitro) at 0.5.
- ⑤ Repeat using conditioned media

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 22004

ARC USE ONLY

PO. # _____
 ORDER DATE _____
 REF. # _____
 CONTACT _____
 EST AMT _____
 SCHED DEL _____
 SPECIAL ROUTING _____
 NON COM VENDOR _____
 FLAGGED BY PROTOCOL _____

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASIS
 REQUEST BY: Dr. Greg Sibley DATE: 11/19/94
 REQUESTORS PHONE NUMBER: 2-0294
 AUTHORIZED SIGNATURE: [Signature]

FAS ACCOUNT 6-95150-5100 PI: H. Hahen
 VENDOR: FCR1 PROTOCOL: 58671
 REQUESTED DELIVERY DATE: 11/17/94 PHONE: 2-6819

SPECIES: mouse QUANTITY: 48
 STRAIN: Athymic Nude SEX: M (F) EITHER
 WEIGHT/AGE: 5-6wks ALTERNATE WEIGHT/AGE: _____
 (IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT
 AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
 (RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER X CLSC _____ FMI _____ OTHER _____

11/4/94-1

EFFICIENCY LINE 22-206

Purpose -

- Establish dose response survival curve with V87 cell line & 899-6 virus @ 10^{-1} & 10^{-3}

Design -

- Fixed variables - cell type V87

- intervals - infect $\times 2^{\circ} \rightarrow$ subcult $\rightarrow 4^{\circ} \rightarrow$ XRT

- Study variables - virus MOI = 10^{-1} & 10^{-3} 899-6

- RT dose : 0, 2, 3, 5, 7, 9 Gy

11/4/94 - 2

EFFICIENCY LINE # 22-206

METHODS -

11/4/94 ① Plate V87 cells on 3 60mm dishes = 5×10^5
 11/10/94 ② ~~Count~~ Grow to subconfluence, Count 1 dish to
 calculate MOI, Count = 3×10^6 cells

New 899-6 titer = 1.2×10^9 PFU/ml

For MOI 10^{-1} : 3×10^5 PFU / 1.2×10^9 PFU/ml d.i. = ~~0.00025~~ 25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

For MOI 10^{-3} : 3×10^3 PFU / 1.2×10^9 PFU/ml d.i. = ~~2500~~ 25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

③ Infect x 2° then subcult to 100mm plates (in dupl),

RT Virus # plated

0 10^{-1} 1000 2000 5000

2 10^{-1} 5000 10^4

3 10^{-1} 10^4 2×10^4

5 10^{-1} 10^4 2×10^4

7 10^{-1} 5×10^4 10^5

9 10^{-1} 5×10^4 10^5

0 10^{-3} 1000 2000 5000

2 10^{-3} 5000 10^4

3 10^{-3} 10^4 2×10^4

5 10^{-3} 10^4 2×10^4

7 10^{-3} 5×10^4 10^5

9 10^{-3} 5×10^4 10^5

0 0 500 1000 5000

11/28/94 ④ Stain & count (perfect size to count)

RESULTS -

1	2	3	4	5	6	7	8	9
899-6 14125	RT	# Plotted	# Counted		Ave Surv.	P.E.	rel Surv.	
10 ⁻¹	0	1000	0	—	—	.008	1	
		2000	1	1	—			
		5000	23	57	.008			
	2	5000	11	3	.0045		.56	
		10 ⁴	74	47				
	3	10 ⁴	16	10	.0024		.29	
		2x10 ⁴	68	Tm				
	5	10 ⁴	0	5	.002		.25	
		2x10 ⁴	55	24				
	7	5x10 ⁴	61	81	.0014		.18	
		10 ⁵	Tm	Tm				
	9	5x10 ⁴	17	25	.0004		.05	
		10 ⁵	84?	Tm				
10 ⁻³	0	1000	2	0	.008 .0067	.008 .0067	1	
		2000	7	9				
		5000	43	38				
	2	5000	4	3	.0058		.71	
		10 ⁴	60	55				
	3	10 ⁴	37	50	.0044		.54	
		2x10 ⁴	Tm	Tm				
	5	10 ⁴	10	21	.0026		.32	
		2x10 ⁴	63	62				
	7	5x10 ⁴	98	97	.002		.24	
		10 ⁵	Tm	Tm				
	9	5x10 ⁴	34	39	.0007		.09	
		10 ⁵	Tm	Tm				

V87 - TNF

11/9/44 - 1

EFFICIENCY LINE 22-206

Purpose - Quantify TNF production in V87 cell line
with 899-6 virus #1 - 5 C_q RT.
Use 3616 virus as control

Design -

- Fixed variables - Cell type V87

- intervals = infect x 2 hrs → RT

- aliquot media @ 0, 2, 4, 6 hrs then
subculture & save 10⁶ cells

- Study variables - virus: None, 3616, 899-6 @ MOI=10⁻
RT: 0 C_q, 5 C_q

Conclusion - 10⁵ PFU too little to show TNF production c.
899-6

11/9/94-2

METHODS -

11/11/94 ① Plate V87 cells in 60 mm dishes.

Grow to subconfluent. Need ~~20~~¹⁵ dishes

11/13/94 ② Count 1 dish to determine # PFU needed for
mol of $10^{-1.5}$ No. Infect $\approx 10^5$ PFU (use

this also for other cell lines to compare TNF production
i.e. TNF production should be dependent only on PFU &
not on cell number (mol not important)).

$$899-64 \text{ titer} = 1.2 \times 10^9 \text{ PFU/ml} : \frac{10^5 \text{ PFU}}{1.2 \times 10^9 \text{ PFU/ml}} = 0.83 \text{ ml of } 1.2 \times 10^5 \text{ d.i./l (+ .17 media)}$$

$$3616 \text{ titer} = 2.3 \times 10^9 \text{ PFU/ml} : \frac{10^5}{2.3 \times 10^9 \text{ PFU/ml}} = 0.43 \text{ ml " } 2.3 \times 10^5 \text{ " (+ .57 media)}$$

③ Infect cells $\times 2^\circ$ in 1 ml 199K media.

Add

④ Remove inoculum & add 6 ml of 10% FCS media
(V87 media) + IgG.

⑤ Irradiate $\approx 5 \text{ Gy}$

⑥ Aliquot 1 ml media @ 0, 2, 4, 6^o & subculture in 5n
~~to 10^6 cells/ml~~ \rightarrow save as 1 ml as "pellet".

⑦ Centrifuge aliquots before using for TNF ELISA assay

⑧ To calculate total TNF:

$$899-6 = ([0^\circ] \times 1 \text{ ml}) + ([2^\circ] \times 1 \text{ ml}) + ([4^\circ] \times 1 \text{ ml}) + ([6^\circ] \times 1 \text{ ml}) + ([6^\circ \text{P}] \times 2 \text{ ml})$$

$$3616 = ([6^\circ] \times 6 \text{ ml}) + ([6^\circ \text{P}] \times 2 \text{ ml})$$

$$0 = ([6^\circ] \times 6 \text{ ml}) + ([6^\circ \text{P}] \times 2 \text{ ml})$$

V-87-899-6

11/16/94 - 1

EFFICIENCY LINE 22-206

Purpose - Generate Cell Survival Curve for V-87 cells
with different Mol's of 899-6 virus.

Design - Fixed Variables - Cell Type V87
- intervals = infect x 2°
- Virus = 899-6

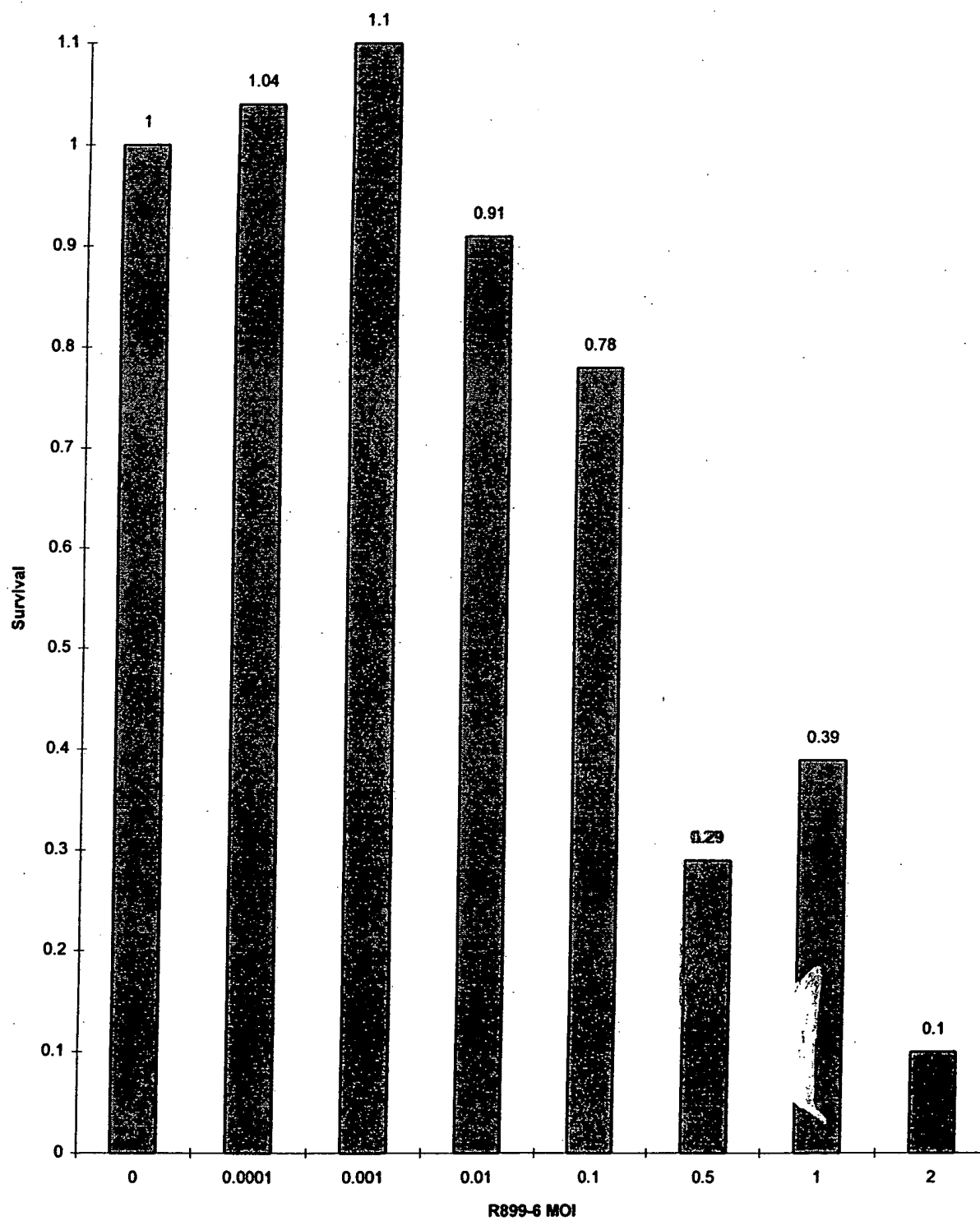
Study Variables = Mol = 2, 1, .5, 10^{-1} , 5×10^{-2} , 10^{-2} ,
 5×10^{-3} , 10^{-3} , 10^{-4}

11/16/84-3

11/16/84-3

[illegible]

Survival By R899-6 MOI



U-87

11/16/94 - 4

	1	2	3	4	5	6	7	8	9	
1										
2	<u>CONCLUSIONS -</u>									
3	① R89A-6 doesn't appear to have an appreciable effect									
4	until MOI ~ 0.1									
5	② ? TCD ₅₀ ~ MOI = 0.5									
6	③ Repeat using MOI's of 0, 10 ⁻² , 10 ⁻¹ , 0.5, 1, 2,									
7	3, 5.									
8	④ Plating efficiency still very low (2%), try									
9	using conditioned media (50/50)									
10										
11										
12										
13										
14										
15										
16										
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EFFICIENCY LINE® 22-206



2.2×10^8 photons

[illegible]

[TNF] By Virus Type (10 exp5 PFU) and RT Dose in U-87 Cells

Virus	Dose	Time	[TNF]	Total [TNF]
899-6	0	0	0.19	62.52
899-6	0	2	2.34	
899-6	0	4	4.99	
899-6	0	6	11.04	
899-6	0	p	10.94	
899-6	5	0	0.94	64.37
899-6	5	2	1.49	
899-6	5	4	4.99	
899-6	5	6	12.29	
899-6	5	p	10.04	
None	0	6	6.64	42.62
None	0	p	1.39	
None	5	6	3.64	24.42
None	5	p	1.29	
R3616	0	6	5.44	42.92
R3616	0	p	5.14	
R3616	5	6	11.09	
R3616	5	p	5.34	

RAY : _____

DATE: _____

OPERATOR: _____ O.D. LIMIT: 3.000

TIME: _____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY

AUTO MIX: ON

NOTES: _____ CAL: ON

OPTICAL DENSITY

	25	26	27	28	29	30	31	32	33	34	35	36
A	0.063	0.176	0.073	0.066	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.081	0.070	0.035	0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.067	0.076	0.080	0.077	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.068	0.074	0.030	0.113	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.073	0.079	0.035	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.101	0.077	0.106	0.158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.076	0.068	0.036	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.063	0.070	0.031	0.083	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000

$$y = .0643 / .005$$

$$y = .005x + .0643$$

$$y = .0657 e^{.6604x}$$

$$\ln y - \ln .0657 = .6604x$$

MOLECULAR DEVICES

Kinetic Plate Reader with Microplate Reader

PLATE #: 1

ID#

V87

DATE: 11.17.94

OPERATOR: Greg

O.D. LIMIT: 2.000

TIME: : : AM/PM

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: ON

NOTES:

CAL: ON

Standards

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.086	0.097	0.101	0.092	0.093	0.080	0.073	0.080	0.072	0.127	0.101
B	0.129	0.144	0.070	0.072	0.375	0.566	0.272	0.235	*	*	0.094	0.091
C	0.199	0.198	-	-	0.737	0.395	0.420	0.521	1.311	1.202	0.366	0.317
D	0.371	0.367	0.343	0.342	0.088	0.086	0.161	0.143	1.578	1.442	0.383	0.070
E	0.670	0.727	0.154	0.153	0.297	0.288	-	-	1.205	1.191	0.127	0.140
F	1.333	1.385	2.660	0.680	0.647	0.635	0.149	0.110	2.623	2.650	0.198	0.166
G	2.607	2.460	-	-	1.771	1.877	0.129	0.062	0.726	0.700	0.121	0.116
H	-	-	0.203	0.143	2.161	2.483	0.086	0.063	0.131	0.039	0.600	0.629

MOLECULAR DEVICES

Kinetic Plate Reader with Microplate Reader

PLATE #: 1

ID#

DATE: : : AM/PM

OPERATOR:

O.D. LIMIT: 2.000

TIME: : : AM/PM

WAVELENGTH: 450nm - OPT 2

READ MODE: OPTICAL DENSITY

AUTO MIX: ON

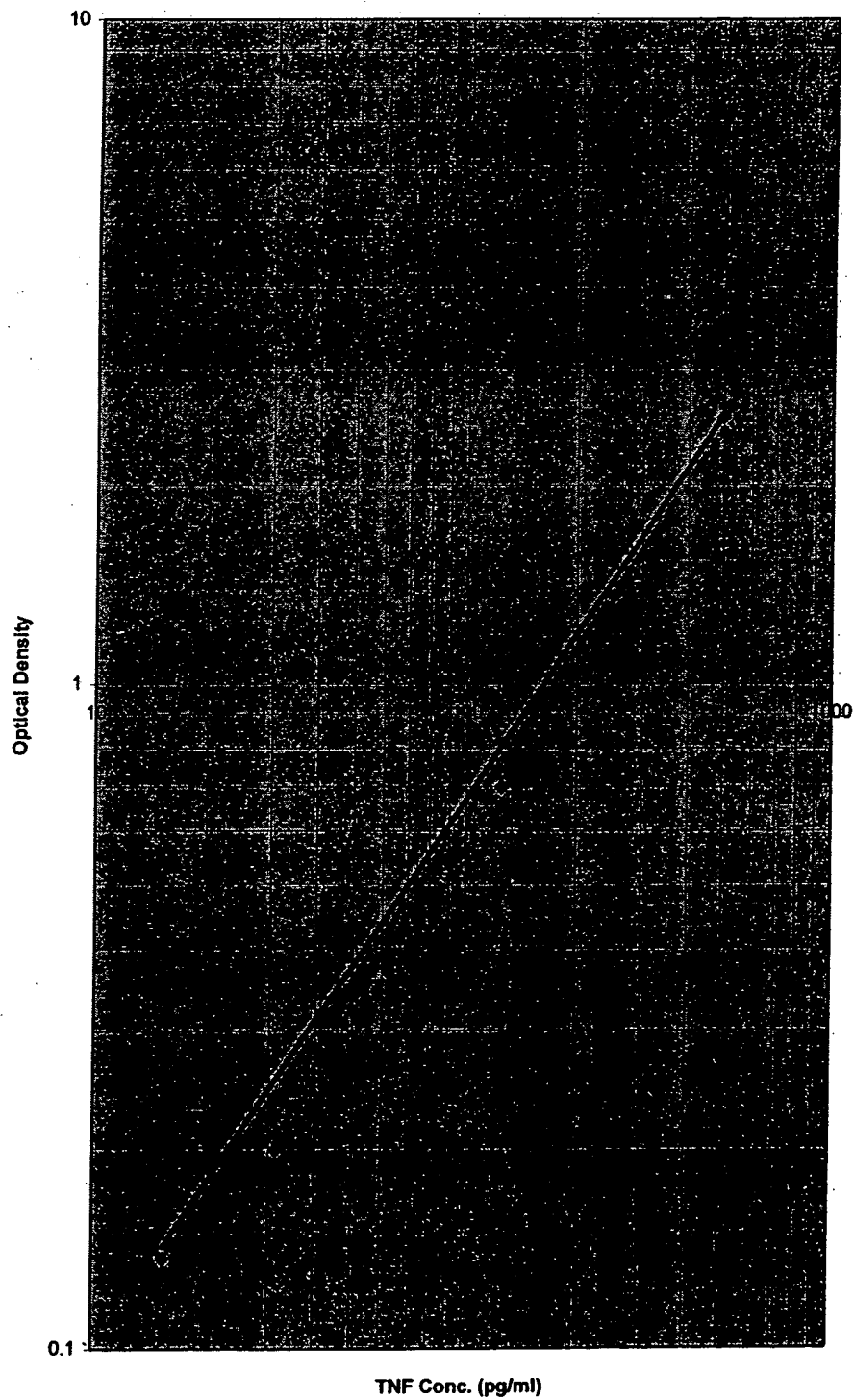
NOTES:

CAL: ON

OPTICAL DENSITY

	13	14	15	16	17	18	19	20	21	22	23	24
A	0.075	0.070	0.199	0.188	0.327	0.324	0.151	0.152	0.097	0.087	0.077	0.070
B	0.225	0.267	0.148	0.180	0.137	0.151	0.106	0.109	0.093	0.083	0.072	0.062
C	0.810	0.841	0.478	0.609	0.074	0.075	0.219	0.212	0.127	0.117	0.066	0.032
D	0.250	0.263	0.340	0.255	0.128	0.125	0.064	0.059	0.114	0.120	0.086	0.031
E	0.583	0.716	0.073	0.082	0.043	0.037	0.055	0.060	0.109	0.122	0.120	0.125
F	0.124	0.134	0.123	0.183	0.042	0.043	0.064	0.078	0.124	0.121	0.130	0.126
G	0.271	0.242	0.098	0.102	0.030	0.032	0.065	0.073	0.067	0.071	0.109	0.112

TNF ELISA Plot 11/17/94



V-87
R899-6

11/29/94-1

EFFICIENCY LINE 22-206

PURPOSE - Generate cell survival curve for V-87 cells
with different MOI's of 899-6 virus

Design - Fixed variables - cell type V-87
- intervals = infect x 2°
- virus 899-6

Study variables - MOI: $0, 10^{-2}, 10^{-1}, 0.5,$
 $1, 2, 3, 5$

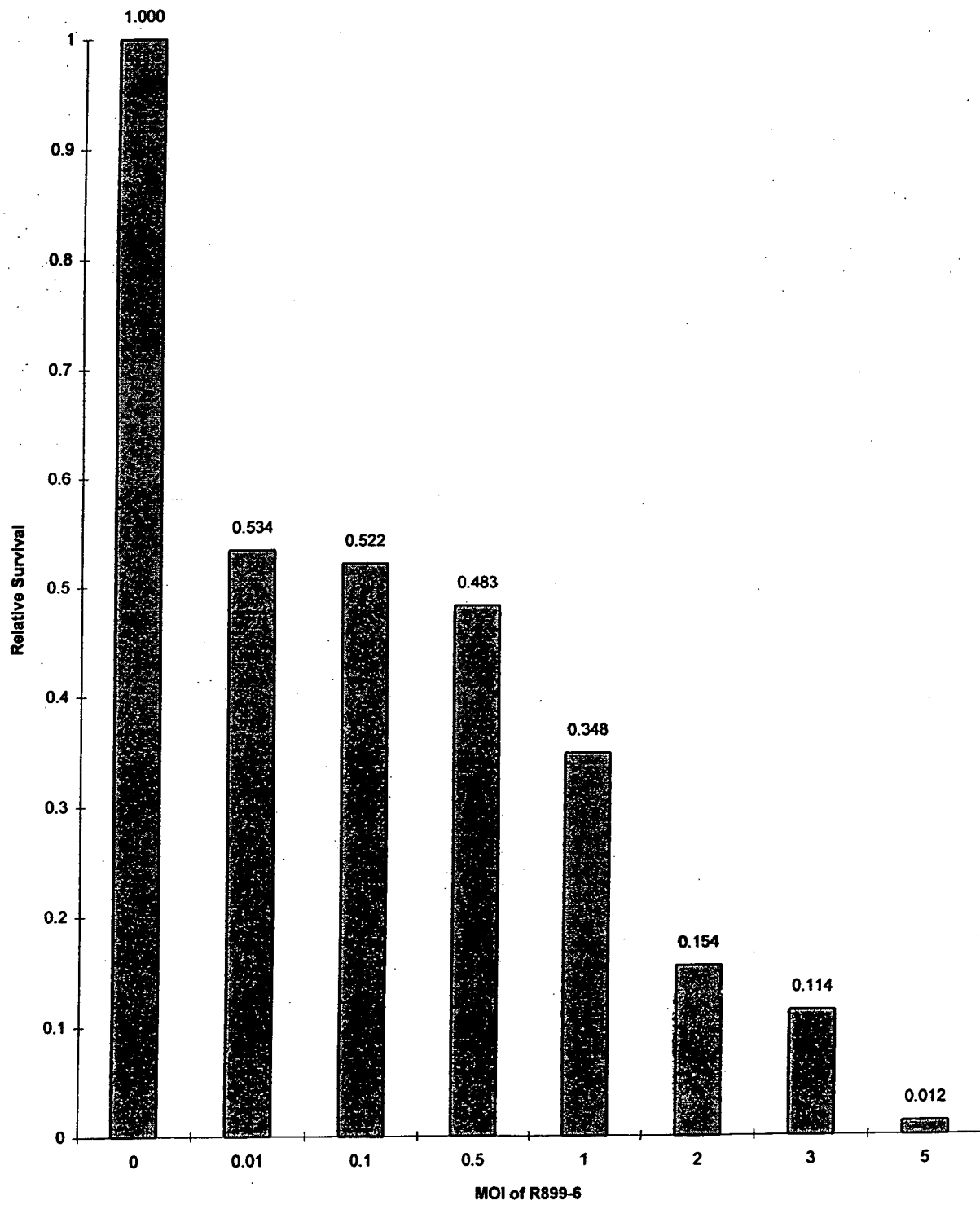
11/29/94 - 3

[illegible]

11/29/94: Survival of U-87 Cells by MOI of R899-6

MOI	# plated	# counted			Ave. Surv.	P.E.	Rel. Surv.
0	2000	21	12	19	0.0107	0.0107	1
	5000	68	64	59			
0.01	2000	5	6	6	0.005717	0.0107	0.534268
	5000	53	28	48			
0.1	2000	4	11	6	0.005583	0.0107	0.521807
	5000	30	50	35			
0.5	5000	34	22	21	0.005167	0.0107	0.482866
	10000	51	70	35			
1	10000	36	37	24	0.003725	0.0107	0.348131
	20000	90	83	80			
2	10000	18	13	17	0.00165	0.0107	0.154206
	20000	33	25	44			
3	20000	18	17	14	0.001215	0.0107	0.113551
	50000	69	84	89			
5	50000	7	8	5	0.000133	0.0107	0.012461
MOI	Rel. Surv.						
0	1.000						
0.01	0.534						
0.1	0.522						
0.5	0.483						
1	0.348						
2	0.154						
3	0.114						
5	0.012						

Relative Survival By R899-6 MOI in U-87 Cells



V-87 Mycoplasma assay

12/6/94

EFFICIENCY LINE 22-206



PURPOSE - Rule out mycoplasma infection as reason for poor plating efficiency in V87 cells

METHODS - see attached protocol

CONCLUSION - No mycoplasma infection

12/12/94-1

12/12/94-1

Purpose - Quantity TAT production in U87 cell line
 \bar{c} R3616, 899-6 & 0 virus \bar{c} 0 or 9 Gy
 (mol of virus = 0.5) Use 2×10^6 PFU (umol = 1)

METHODS -

① Plate U87 cells in 60 mm dishes & grow to subconfluence.

② 894-6: $2 \times 10^6 \text{ PFU} / 1.2 \times 10^7 \text{ PFU/ml} = .17 \text{ ml}$

$$3616 : 2 \times 10^6 \text{ PK} / 2.3 \times 10^6 \text{ PF-U/ml} = .87 \text{ ml}$$

③ Infect $\times 2^0$ in total volume / cc 199V media

③ Remove inoculum & add 6 ml of 10% FCS media

~~10~~ Diquat 1 ml med. @ 2, 6

④ Irradiate 0 9 6 RT, 11 Am 9 pm 9 Am 3 pm

(5) Alizvoť 1 ml med'ia @ 2°, 6°, 12°, 24°, 30°

then subculture ^{in 3 ml} and gave 1 ml as pellet

26

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TNF ELISA Assay: U-87 Cells Infected with R899-6, R3616, or No Virus (MOI=0.5) +/- 9 Gy RT (12/7/94)

Standards

reading 1	reading 2	[TNF]	ave. reading
0.087	0.087	15.6	0.087
0.119	0.137	31.3	0.126
0.205	0.217	62.5	0.211
0.39	0.407	125	0.399
0.733	0.756	250	0.745
1.354	1.459	500	1.407
1.997	2.284	1000	2.141

				Virus	Dose	Time	Total [TNF]
0.044	0.044	7.8	0.044	None	0	0	62.60
0.045	0.046	8.1	0.046	None	0	10	
0.04	0.046	7.6	0.043	None	0	21	
0.044	0.042	7.6	0.043	None	0	27	
0.043	0.047	8.0	0.045	None	0	P	
0.04	0.048	7.8	0.044	None	9	0	67.73
0.051	0.046	8.8	0.049	None	9	10	
0.052	0.054	9.8	0.053	None	9	21	
0.051	0.048	9.0	0.050	None	9	27	
0.043	0.045	7.8	0.044	None	9	P	
0.047	0.041	7.8	0.044	R3616	0	0	72.24
0.047	0.044	8.1	0.046	R3616	0	10	
0.042	0.055	8.8	0.049	R3616	0	21	
0.054	0.059	10.6	0.057	R3616	0	27	
0.05	0.047	8.8	0.049	R3616	0	P	
0.046	0.042	7.8	0.044	R3616	9	0	67.48
0.046	0.04	7.6	0.043	R3616	9	10	
0.051	0.05	9.2	0.051	R3616	9	21	
0.046	0.048	8.4	0.047	R3616	9	27	
0.048	0.048	8.7	0.048	R3616	9	P	
0.054	0.05	9.6	0.052	R899-6	0	0	3004.23
1.476	1.587	633.2	1.532	R899-6	0	10	
1.836	1.48	698.6	1.658	R899-6	0	21	
1.752	1.973	806.9	1.863	R899-6	0	27	
0.08	0.08	16.3	0.080	R899-6	0	P	
0.047	0.051	8.9	0.049	R899-6	9	0	3323.99
0.939	0.829	320.5	0.884	R899-6	9	6	
1.503	1.569	635.5	1.536	R899-6	9	10	
1.83	1.96	824.4	1.895	R899-6	9	21	
2.076	1.99	899.4	2.033	R899-6	9	27	
0.09	0.089	18.8	0.090	R899-6	9	P	

V-87 ~~2711~~
R3616

12/12/94

EFFICIENCY LINE 22-206



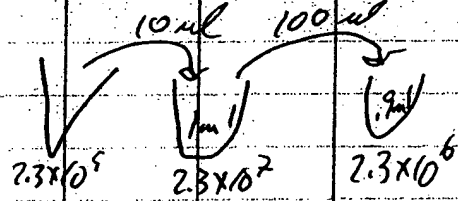
PURPOSE - Survival of V87 cells \bar{c} ~~RT~~ ^{R3616} @ MOI of 0 (control) & 0.5 \bar{c} RT doses: 0, 3, 5, 7, 9 Gy

METHODS -

- ① Plate 10^6 cells in 60 mm dish (12/1) & grow to confluence
- ② Cant a dish & infect ~~one~~ one dish \bar{c} MOI 0.5 of ~~RT~~ R3616
- 12/13/94 ③ Infect x 2° in 1 ml 199V @ 37°
- ④ Subculture into 100 mm plates \bar{c} 5% Cond. Medium (+ IgG). Wait 6.
- ⑤ Irradiate @ 0, 3, 5, 7, 9 Gy
- ⑥ Incubate x 3 wks
- ⑦ Stain & Count
- ⑧ Aliquot media ~~@ 30~~ @ 1 wk for [TNF]
- (? is there induction \bar{c} 7 doses RT)

Dish count = 2.3×10^6 cells

R3616 titer = 2.3×10^9 PFU/ml



Use 0.5 cc 2.3×10^6 dilution = MOI 0.5

12/12/84

[illegible]

V-87
R899-6
12/8/94-1

EFFICIENCY LINE 22-206

Purpose - Generate cell survival curve for V-87 cells
with RT doses 0, 5, 9 Gy, # mol's of 899-6 =
0.5, 0.1, 0

METHODS ① Plate 10^6 cells per 60 mm dish = V-87

② When subconfluent (2 days) infect c. virus in
1 cc 1991 x 2° .

not Titer R899-6 = 1.2×10^9 pfu/ml
mol = 1 = $\frac{1.6 \times 10^6}{1.2 \times 10^7} = 0.133$

mol = 1.5 = $\frac{0.8 \times 10^6}{1.2 \times 10^6} = 0.667$

~~mol = 0.1 = $\frac{1.6 \times 10^5}{1.2 \times 10^6} = 0.133$~~

③ Subculture into 10 mm dishes at appropriate concentration
c. 50% conditioned media.

④ Let cells adhere x 6°

⑤ Irradiate

⑥ Incubate x 3 wks, stain & count.

Diagram showing virus titration:
V (1.2x10⁹) → 100% → 1.2x10⁷ → 1.2x10⁶ → 1.2x10⁵ → 1.2x10⁴ → 1.2x10³ → 1.2x10² → 1.2x10¹ → 1.2x10⁰

12/18/94-2

[illegible]

12/20/94

PURPOSE - Assess toxicity of R899-6 & R3616 in nude mice

METHODS -

① 8 mice

R899-6: 10 μ l of 1.2×10^8 PFU/ml = 1.2×10^7 PFU (2 mice)
 10 μ l of 1.2×10^8 PFU/ml = 1.2×10^6 PFU (2 mice)

R3616: 10 μ l of 2.3×10^9 PFU/ml = 2.3×10^7 PFU (2 mice)
 10 μ l of 2.3×10^8 PFU/ml = 2.3×10^6 PFU (2 mice)

Cage AA104551 - { 1681 \emptyset R899-6 1.2×10^7
 1682 L " "
 1683 R " 1.2×10^6
 1684 LR " "

Cage AA104552 - { 1685 \emptyset R3616 2.3×10^7
 1686 L " "
 1687 R " 2.3×10^6
 1688 LR " "

② Injected 3⁰⁰pm 1/5/94

RESULTS -

No local or systemic effects noted
 Mice examined twice weekly for weeks
 Said on

1/9/95

EFFICIENCY LINE 22-206

	1	2	3	4	5	6	7	8	9
PURPOSE -	To improve plating efficiency of V87 cell line by trying different media								
METHODS -									
Base media	% FCS	CM	# plated	# counted					
MEM/Npyr/aa	10%	+	1000	infected	infected				
"	10	+	5000	infected	infected				
"	15	+	1000	159	infected				
"	15	+	5000	infected	infected				
"	20	+	1000	infected	infected				
"	20	+	5000	tm	infected				
DME - F12	10%	+	1000	infected	infected				
"	10	+	5000	tm	tm				
"	15	+	1000	91	infected				
"	15	+	5000	tm	tm				
"	20	+	1000	131	50				
"	20	+	5000	tm	infected				

January 10, 1995

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

As per our telephone conversation on 1/10/95, please place the following mice on TMP-SMZ (Bactrim) for 1 week:

Cages AA105283, AA104542 through AA104545, AA104547 through AA104550,
and AA107678 through AA107710.

This should include all mice from Hallahan's Lab (Radiation Oncology) currently in Cummings Room #1053.

Thank you. If you have any questions you can contact me at beeper #3439 or contact Helena at 2-0294.

Sincerely,

Gregory S. Sibley, M.D.

REQ NO 18777

ARC USE ONLY

P.O. # _____
ORDER DATE _____
REF. # _____
CONTACT _____
EST AMT _____
SCHED DEL _____
SPECIAL ROUTING _____
NON COM VENDOR _____
FLAGGED BY PROTOCOL _____

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASIS
REQUEST BY: Gregory Sibley MD DATE: 1/16/95
REQUESTORS PHONE NUMBER: 2-0294
AUTHORIZED SIGNATURE: Mohamed R. Sibley
FAS ACCOUNT: 2-73731-5100 PI: H. H. Hagan
VENDOR: FCR 1 PROTOCOL: 58671
REQUESTED DELIVERY DATE: 1/23/95 PHONE: 2-6849 2-0294

SPECIES: Mouse QUANTITY: 60
STRAIN: Athymic Nude SEX: M ☒ F EITHER
WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: _____
(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

HOUSE AT: _____ CARLSON _____ WYLER _____ CLSC _____ FMI _____ OTHER _____

PROCUREMENT DESK: 2-9364

V87 TWF (intervals)

1/17/95

EFFICIENCY LINE 22-206

PURPOSE - To determine optimal interval from infect (R899-6) to RT (9 Gy) to optimize TWF production in V87 cells

METHODS -

1/16/95 ① Plate 10^6 V87 cells per 60 mm dishes. Incubate ~ 36° for ~ 2×10^6 cells

1/17/95 ② Infect plates with 10^6 PFU of R899-6 at
1/18/95 12° prior to RT = 10^{PM}
 8° prior to RT = 2^{AM}
 4° " " " = 6^{AM} (Actually $6^{\text{45}} = 3.25$ hrs,
 2° " " " = 8^{AM}

Titer R899-6 = 1.2×10^9 PFU/ml

\therefore Use 0.83 ml of 1.2×10^6 PFU/ml dilution + 199V media to total vol = 1.5 cc

③ Add 2.5 ml 10% FCS media + IgG (total vol 4 cc, "Control" dishes \Rightarrow Aliquot 1 ml as "supernatant"
Try Decant remaining media
Trypsinize cells \bar{c} 1 ml trypsin + 1 ml Verce
Then add 2 ml media (total vol = 4.
Aliquot 1 ml as "pellet"

④ For "Treatment" dishes RT \bar{c} 9 Gy
Wait 7° then repeat aliquot, done for
Supernatant & pellet as above

TNF ELISA 1/20/95 U-87 Cells

reading 1	reading 2	average	[TNF]	Time	Sample	Average	Total [TNF]	Rel. [TNF]
1.267	1.308	1.2875	325.978	12 hours	control-super	1283.22	1623.47	1239.04
1.248	1.256	1.252	315.632	12 hours	control-super			
0.38	0.353	0.3665	76.5162	12 hours	control-pellet	340.25		
0.474	0.399	0.4365	93.6086	12 hours	control-pellet			
2.331	2.31	2.3205	643.123	12 hours	9 Gy-super	2394.87	2862.506	
2.053	2.027	2.04	554.311	12 hours	9 Gy-super			
0.505	0.534	0.5195	114.425	12 hours	9 Gy-pellet	467.638		
0.537	0.541	0.539	119.394	12 hours	9 Gy-pellet			
0.417	0.422	0.4195	89.416	8 hours	control-super	360.864	642.5502	2071.4
0.421	0.431	0.426	91.016	8 hours	control-super			
0.311	0.34	0.3255	66.73	8 hours	control-pellet	281.686		
0.343	0.37	0.3565	74.113	8 hours	control-pellet			
1.455	1.503	1.479	382.519	8 hours	9 Gy-super	1953.96	2713.952	
2.127	2.208	2.1675	594.462	8 hours	9 Gy-super			
0.587	0.607	0.597	134.332	8 hours	9 Gy-pellet	759.989		
0.957	1.058	1.0075	245.662	8 hours	9 Gy-pellet			
0.065	0.058	0.0615	9.76247	3.25 hours	control-super	40.8921	130.1639	1371.14
0.065	0.068	0.0665	10.6836	3.25 hours	control-super			
0.085	0.087	0.086	14.3726	3.25 hours	control-pellet	89.2718		
0.158	0.17	0.164	30.2632	3.25 hours	control-pellet			
0.933	0.991	0.962	232.909	3.25 hours	9 Gy-super	906.04	1501.304	
0.901	0.931	0.916	220.111	3.25 hours	9 Gy-super			
0.709	0.714	0.7115	164.467	3.25 hours	9 Gy-pellet	595.264		
0.599	0.586	0.5925	133.165	3.25 hours	9 Gy-pellet			
0.059	0.068	0.0635	10.1296	2 hours	control-super	41.0714	131.0586	2360.16
0.065	0.065	0.065	10.4061	2 hours	control-super			
0.067	0.112	0.0895	15.0494	2 hours	control-pellet	89.9873		
0.179	0.146	0.1625	29.9442	2 hours	control-pellet			
1.414	1.524	1.469	379.538	2 hours	9 Gy-super	1679.41	2491.216	
1.687	1.785	1.736	460.167	2 hours	9 Gy-super			
0.859	0.824	0.8415	199.593	2 hours	9 Gy-pellet	811.806		
0.866	0.866	0.866	206.311	2 hours	9 Gy-pellet			

V-87 TNF (intervals)

1/23/95

PURPOSE - To determine optimal interval from infect (R899-6) to RT (9 Gy) to optimize ~~the~~ subsequent TNF induction experiments

METHODS -

1/23/95 ① Plate 10^6 V87 cells per 60 mm dish. Incubate ~~at~~ 36° for $\sim 2 \times 10^6$ cells

② Infect plates \bar{c} 10^6 PFU R899-6 = 0.83 ml of 1.2×10^6 PFU/ml dilution + 199V media to \bar{c} 1.5 ml

Infect @ 7^{30}_{AM}

③ Aliquot & trypsinize "control dishes" @
 4° \bar{p} infection 11^{30}
 6° " " 1^{30}
 8° " " 3^{30}

④ Irradiate @ same time points \bar{c} 9 Gy

⑤ Aliquot & trypsinize "treatment dishes" @
 7° \bar{p} RT = 6^{30}_{PM} , 8^{30}_{PM} , 10^{30}_{PM}

In Vivo - TNF induction - intervals
U87 hind limbs nude mice

1/24/95

PURPOSE - To determine optimal interval from infection \rightarrow RT
in U87 tumors in hind limbs of nude mice for TNF
production

METHODS -

① Transplant 1mm^3 U87 tumors into hind limbs of
nude mice. Grow to $\sim 6\text{mm}$ (range 4.5-8 mm)
greatest dimension.

② Infect mice in groups of 4 @ 48° , 24° , 10° & 5°
prior to RT.

- $48^\circ = 4\text{pm } 1/24$

- $24^\circ = 4\text{pm } 1/25$

- $10^\circ = 7\text{am } 1/26$

- $5^\circ = 12\text{N } 1/26$

$0^\circ = 5\text{pm } 1/26 = \text{Irradiate } \pm 20\text{ Gy single fraction.}$

③ Sacrifice mice @ 4pm

1/24/95

Greg's Mouse Log

Mouse #	Cage #	DOB	Tumor Date	Infect Date	RT Date	Date Sacrif	Tumor size	
							1/23/95	1/24
1703	AA104542	10/10/94	1/10/95	R ?			7.5	7x5x3.5
1711	AA104542	10/10/94	1/10/95	Ø } OK			6	5.5x5x2.5
1712	AA104542	10/10/94	1/10/95	L } OK			8	10x8x6
1714	AA104542	10/10/94	1/10/95	LR			6	6x6x3.5
1701	AA104543	10/10/94	1/10/95	Ø			6.5	7.5x6x4
1715	AA104543	10/10/94	1/10/95	Ø → L			4.5	5.5x5x3
1718	AA104543	10/10/94	1/10/95	LR			5.5	4x5.5x2.5
1721	AA104543	10/10/94	1/10/95	Ø → R			7	6x7.5x3
1716	AA104544	10/10/94	1/10/95				3.5	
1722	AA104544	10/10/94	1/10/95				3.5	
1723	AA104544	10/10/94	1/10/95				3	
1724	AA104544	10/10/94	1/10/95				2	
1689	AA104546	10/10/94	12/6/94			1/10/95		
1690	AA104546	10/10/94	12/6/94			1/10/95		
1691	AA104546	10/10/94	12/6/94			1/10/95		
1692	AA104546	10/10/94	12/6/94			1/10/95		
1693	AA104547	10/10/94	12/6/94	Ø			10	
1694	AA104547	10/10/94	12/6/94			1/24/95	20	
1695	AA104547	10/10/94	12/6/94	SAC			0	
1696	AA104548	10/10/94	12/6/94	LR			6	6.5x5.5x3.5
1698	AA104548	10/10/94	1/10/95	L			4	9x9.5x8
1699	AA104548	10/10/94	1/10/95	R			5	6x5x3
1700	AA104548	10/10/94	1/10/95	LR → LRR			6	4.5x2.5x2
1697	AA104549	10/10/94	1/10/95				3.5	
1702	AA104549	10/10/94	1/10/95				2	
1713	AA104549	10/10/94	1/10/95				4.5	
1717	AA104549	10/10/94	1/10/95				2.5	
1704	AA104550	10/10/94	1/10/95				3.5	
1705	AA104550	10/10/94	1/10/95			1/10/95		
1706	AA104550	10/10/94	1/10/95	SAC		1/24/95	0	
1719	AA104550	10/10/94	1/10/95				3.5	
1720	AA104550	10/10/94	1/10/95				3.5	
1681	AA104551	10/10/94	n/a	1/5/95				
1682	AA104551	10/10/94	n/a	1/5/95				
1683	AA104551	10/10/94	n/a	1/5/95				
1684	AA104551	10/10/94	n/a	1/5/95				
1685	AA104552	10/10/94	n/a	1/5/95				
1686	AA104552	10/10/94	n/a	1/5/95				
1687	AA104552	10/10/94	n/a	1/5/95				
1688	AA104552	10/10/94	n/a	1/5/95				
1707	AA105283	10/10/94	1/10/95				0	
1708	AA105283	10/10/94	1/10/95				3	
1709	AA105283	10/10/94	1/10/95				2	
1710	AA105283	10/10/94	1/10/95				3	

24°

8°

48°

3/4 8970 Control

1861	23	20.5	12	25.1
1868	23	21	20.5	22.3
869	21	23	15	24.2
1870	20	18	13	26.9

4550 Control

1719	20	11.5	10	21.8
1722	21	23	19	25.9
1717	17	15.5	12	23.1

5283 891-6

1720	12.5	11.5	3	20.7
1743	0	0	0	22.0
1720	11.5	11.5	1.5	26.0

4549 3616

1697	14.5	12	7.5	26.3
1723	0	0	0	27.1
1709	5.5	6	1.5	25.8
1708	3	3.5	2	24.0

8968 3616

1875	17.5	14.5	7	22.3
1876	14	10.5	10	24.9
1877	11.5	10	11	25.5
1878	15	15	10	22.0

8972 RT

1859	9.5	10	0	22.8
1860	13	11.5	6	19.0
1861	10	12	5	19.1
1862	9	8	3.5	23.3

8964 RT

1892	12.5	12.5	7	23.1
1893	10.5	9.5	7	22.4
1894	9	5.5	5	21.4

8969 3616 + RT

1871	7	7	5	23.7
1872	6	6	4	22.2
1873	5	2.5	5.5	22.2
1874	8.5	7.5	7	26.0

8967 899-6 + RT

1879	3	3	0	24.5
1880	3.5	3	1	20.2
1881	5	6.5	2	23.9
1882	6.5	3.5	3.5	21.7

8971 3616 + RT

1863	9.5	8	3.5	19.7
1864	11	7	3	23.2
1865	7	5.5	3.5	24.4
1866	7	5.5	4	25.3

8960 3616 + RT

1883	4.5	4.5	2.5	20.7
1884	8.5	10	5	20.6
1885	10	8.5	6	19.6
1886	4	4	2	16.5

8968 1073616 427

1875	13.5	12	7	20.6	15.3	14.5	7.5
1876	12	10.5	8	21.3	15.5	16	7.5
1877	10	"	7	23.7	10.5	8.5	7.5
1878	10	8	6	24.3	11	9	7.5

8965 899-6 all mice drowned 2228

1887	9	10.5	6	23.4	wt		
1888	10	7	5.5	24.0			
1889	4	3.5	1	23.4			
1890	6	6	4.5	22.4			

8967 899-6 RT

1879	4	5	3	24.6	wt		
1880	3	3	2	19.5			
1881	7	6	4	22.7			
1882	7	6	4	20.0			

0	0	0
0	0	0
7	6	4
6.5	4.5	3

8969 899-6 RT

1871	8	8	5	20.7	wt		
1872	8	6.5	4.5	20.6			
1873	8.5	9	5.5	20.1			
1874	10	8.5	5	23.6			

7	6	4.5
8	5	4
5	7.5	6
8	7	4

8966 3616 RT

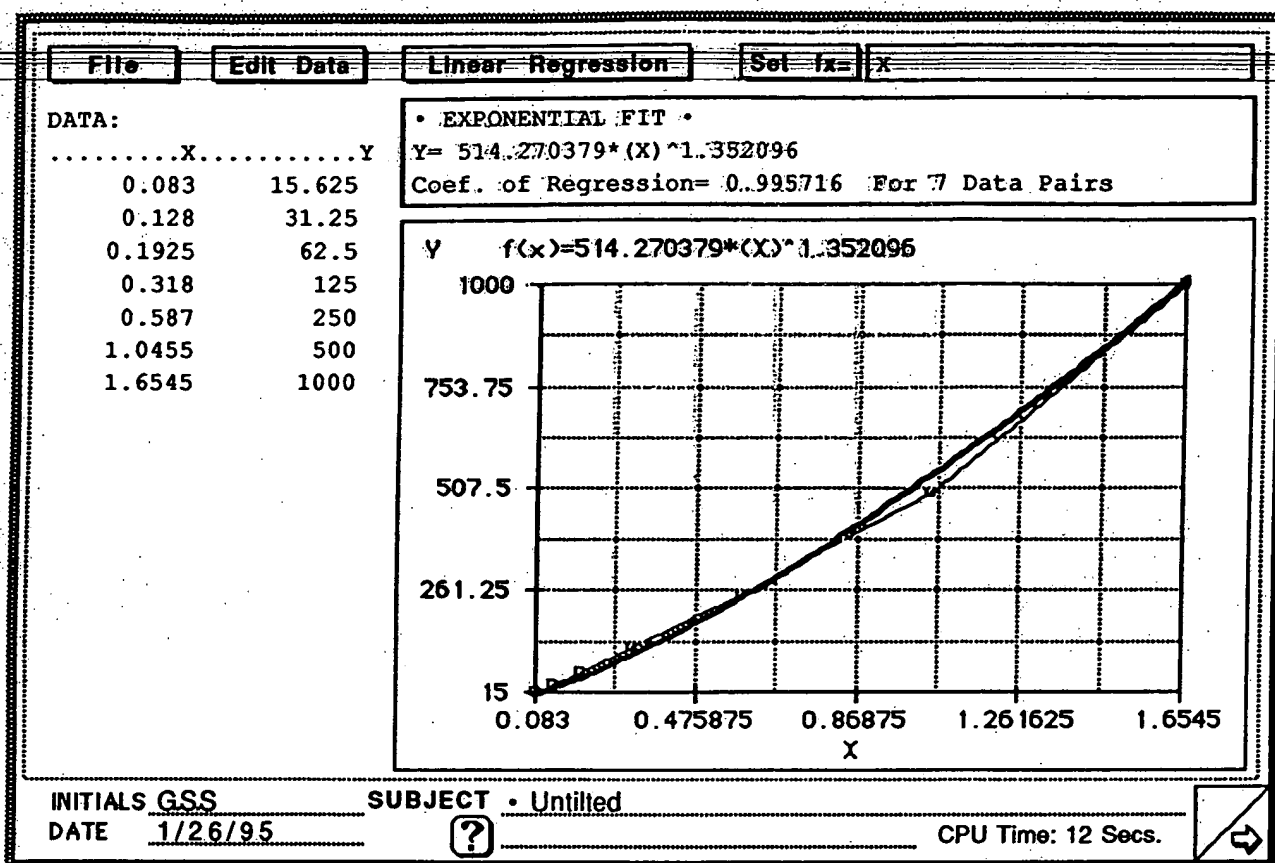
1883	5	4	3.5	20.6	wt		
1884	10	11	7.5	21.1			
1885	11	10	7.5	23.2			
1886	5	6	3.5	19.4			

4	3.5	3.5
11	12.5	6
12.5	9	2.5
4	3.5	2.5

8964 RT

1891	7	7.5	6	20.1	wt		
1892	9.5	9	7	24.0			
1893	9	10	7.5	22.5			
1894	9	9	6	21.8			

7	7	5
9	7.5	6.5
11	9.5	7



TNF ELISA 1/26/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	Time	Sample	[TNF]	Total [TNF]	Rel. [TNF]
0.053	0.063	0.058	10.9483	4 hours	control-super	41.0337	131.644	1497.08
0.053	0.052	0.0525	9.56856	4 hours	control-super			
0.096	0.114	0.105	24.4253	4 hours	control-pellet	90.6103		
0.089	0.098	0.0935	20.8799	4 hours	control-pellet			
0.717	0.721	0.719	329.222	4 hours	9 Gy-super	1275.75	1628.722	
0.646	0.725	0.6855	308.655	4 hours	9 Gy-super			
0.281	0.271	0.276	90.2195	4 hours	9 Gy-pellet	352.969		
0.281	0.253	0.267	86.265	4 hours	9 Gy-pellet			
0.111	0.13	0.1205	29.4229	6 hours	control-super	103.653	305.0815	1957.19
0.09	0.107	0.0985	22.4036	6 hours	control-super			
0.179	0.195	0.187	53.2992	6 hours	control-pellet	201.429		
0.177	0.166	0.1715	47.4151	6 hours	control-pellet			
0.875	0.872	0.8735	428.33	6 hours	9 Gy-super	1776.22	2262.27	
0.889	0.952	0.9205	459.781	6 hours	9 Gy-super			
0.344	0.344	0.344	121.512	6 hours	9 Gy-pellet	486.047		
0.36	0.328	0.344	121.512	6 hours	9 Gy-pellet			
0.305	0.332	0.3185	109.495	8 hours	control-super	307.223	660.3809	1933.52
0.27	0.277	0.2735	89.1164	8 hours	control-super			
0.22	0.21	0.215	64.3647	8 hours	control-pellet	263.158		
0.222	0.222	0.222	67.2141	8 hours	control-pellet			
1.084	1.073	1.0785	569.592	8 hours	9 Gy-super	2237.86	2593.9	
1.068	1.032	1.05	549.337	8 hours	9 Gy-super			
0.28	0.259	0.2695	87.3588	8 hours	9 Gy-pellet	356.041		
0.308	0.246	0.277	90.6617	8 hours	9 Gy-pellet			

1/31/95

[illegible]

YESAY.

507

OPERATOR :

D.B. LIMIT: 2.00

NAME:

Figure 2

WELENGTH: 127mm x 3PT 2

READ MODE: OPTICAL DENSITY

AUTO MIX " 55"

NOTES:

CAL: 1

OPTICAL DENSITY

[illegible]

$$y = 648.842 \times x^{1.84}$$

TNF ELISA 1/31/95 In vivo

reading 1	reading 2	average	[TNF]/ml	Time	Mouse #
0.339	0.207	0.273	59.52191	48 hours	1693
0.117	0.114	0.1155	12.22611	48 hours	1696
1.271	0.47	0.8705	502.7038	48 hours	1699
2.667	1.356	2.0115	2347.548	48 hours	1700
1.744	0.936	1.34	1111.758	24 hours	1703
0.758	0.319	0.5385	207.7396	24 hours	1711
0.328	0.218	0.273	59.52191	24 hours	1712
0.974	0.379	0.6765	316.1043	24 hours	1714
0.85	0.462	0.656	298.7038	8 hours	1701
0.148	0.106	0.127	14.55916	8 hours	1715
1.197	0.631	0.914	549.8935	8 hours	1718
0.572	0.47	0.521	195.4875	8 hours	1721

TNF Interval (Infection with R899-6 to RT with 20 Gy)						
Mouse #	Tumor Size	Mean Size	Interval	[TNF]/ml	Mean [TNF]	[Prot]
1693	342	115.20	48 hours	59.52	730.50	
1696	62.5625		48 hours	12.23		
1699	45		48 hours	502.70		
1700	11.25		48 hours	2347.55		
1703	70	105.59	24 hours	1111.76	423.78	
1711	34.375		24 hours	207.74		
1712	255		24 hours	59.52		
1714	63		24 hours	316.10		
1701	90	56.56	8 hours	298.70	264.66	
1715	27.5		8 hours	14.56		
1718	41.25		8 hours	549.89		
1721	67.5		8 hours	195.49		

Infect \xrightarrow{I} RT $\xrightarrow{24^{\circ}}$ sec $\xrightarrow{20\text{ Gy}}$

$I \xrightarrow{24^{\circ}}$ RT $\xrightarrow{24^{\circ}}$ RT $\xrightarrow{\text{follow}}$

What we have:

- ① 4 mice injected \bar{c} 10^6 V87 cells, now \bar{c} small tumors
- ② 27 mice implanted \bar{c} 1cm^3 V87 implants
- ③ 4 mice 5 tumors injected \bar{c} R3616
- ④ 4 " " " " \bar{c} R899-6

I. Compare 3616 & 899-6 in mice \bar{c} 1° V87 implants

- Design: alternately inject tumors \bar{c} 4×10^7 PFU of R3616 or R899-6 when tumors reach 6 mm in greatest dimension.

Measure tumors 2x/wk

Sac mice (ie. 1 from each group on days 7, 14, 21, 28) for pathology?

II. Use 2° passaged V87 implants (Need 48 mice - order 60)

- Control + Saline

- RT (45Gy single dose) + Saline

- R3616 (4×10^7 PFU single dose)

- R899-6 (4×10^7 PFU single dose)

- RT (45Gy) + R3616

- RT (45Gy) + R899-6

2×10^9
/ ml

0.25

4×10^7

- Design: Take mice in groups of 4 when they reach 6mm in greatest dimension and randomly assign to a group. Use 8 mice per group.

- Deliver RT 24° (?) + viral inoculation

- Measure tumors 2x/wk

1	2	3	4	5	6	7	8	9	
<p>III. In Vivo TNF assay \bar{c} 2^o passaged U87 tumors</p> <ul style="list-style-type: none">- R3616- R899-6- R3616 + RT- R899-6 + RT- Design = inject tumors when reach 6 mm- Sac mice & assay tumors for TNF at specific intervals following inoculation: 12°, 24°, 36°, 48°, 60° (correct for [protein])									
<p>IV. Use 4 mice \bar{c} small tumors inject \bar{c} <u>cells</u> ①</p> <ul style="list-style-type: none">- Sac & assay tumor for TNF to serve as control for III.									

IN VIVO - U87 R899-6, R3616, +/- RT

2/1/95

PURPOSE - To compare effects of R899-6 & R3616 virus, (on U87 xenografts in hind limb of nude mice), with and without RT VS RT alone

METHOD

- 1.) ~~1.)~~ Implant approx 1 mm^3 pieces of ~~U87~~ minced U87 tumor (taken from previous mouse c tumor) into right hind limb of nude mice. Specifically: ① Sac mouse bearing tumor & excise tumor. ② Place in 100 mm tissue culture dish containing culture media & mince into small pieces using scalpel.
- ③ Anesthetize mice with 0.3-0.4 ml of Ketamine/Xylazine preparation
- ④ Paint ③ hind limb with tincture of benzoin
- ⑤ Make small nick in ③ hind limb with #11 blade scalpel
- ⑥ Open subcut space with forceps
- ⑦ Insert tumor implant into pouch c forceps
- ⑧ Apply small steri-strip to close wound
- 2.) Use mice when tumors reach 100 mm^3 (≥ 2 weeks) & randomize to assign to treatment group
- 3.) For all groups: measure tumors twice weekly (M & Th) weigh mice weekly (M) & monitor for adverse eff sac mice when tumors reach , or when sp.
- 4.) RT given on T & Th = 20 Gy (induction dose) & 25
- 5.) Infection delivered v.a 1 injection (3 sites) on Monday (24° prior to first RT dose)

INF 687

2/1/95

PURPOSE - To optimize intervals in order to test for RT inducibility

METHODS -

- ① V87 cells were grown to subconfluence in 60 mm plates
- ② Cells were infected with 10^6 PFU of R899-6 in 1.5 ml 199V media for 2°, then 2.5 ml of V87 media + IgG was added (total vol 4 ml)
- ③ Cells were incubated and irradiated with 8 Gy at the following intervals. The "supernatant" and "pellet" samples were aliquotted as in previous experiments.

Infect @ 6 ^{am} ⇒	RT time	Assay time
	None	6, 8, 10, 12, 14°
	6°	8, 10, 12, 14°
	8°	10, 12, 14°
	10°	12, 14°
	12°	14°

- ④ Prepare samples by freeze thaw x 3, then centrifuge
- ⑤ Measure human TNF α by ELISA

[illegible]

REGULAR DELIVER
 ASSAY: TNF DATE: 2 2 95
 OPERATOR: Sibley/Hyland Q.E. LIMIT: 2.000 TIME: 1 MIN.
 WAVELENGTH: 400nm CF: 2 READ MODE: OPTICAL DENSITY AUTO MIX: OFF
 NOTES: _____ CAL: _____

	OPTICAL DENSITY											
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.041	0.045	0.107	0.105	0.257	0.262	0.700	0.732	0.839	0.850	0.600	0.00
B	0.113	0.118	0.245	0.240	0.128	0.198	0.378	0.409	0.308	0.318	0.308	0.00
C	0.157	0.161	0.227	0.233	0.177	0.230	0.378	0.427	0.317	0.323	0.300	0.00
D	0.070	0.071	0.107	0.102	0.170	0.204	0.311	0.348	0.290	0.303	0.281	0.00
E	0.162	0.163	0.221	0.223	0.217	0.231	0.311	0.343	0.280	0.281	0.280	0.00
F	0.074	0.081	0.111	0.114	0.170	0.198	0.311	0.343	0.280	0.281	0.280	0.00
G	0.177	0.178	0.221	0.223	0.217	0.231	0.311	0.343	0.280	0.281	0.280	0.00
H	0.177	0.178	0.221	0.223	0.217	0.231	0.311	0.343	0.280	0.281	0.280	0.00

$$y = 250.46 * x^{1.1968}$$

File

Edit Data

Linear Regression

Set fx= X

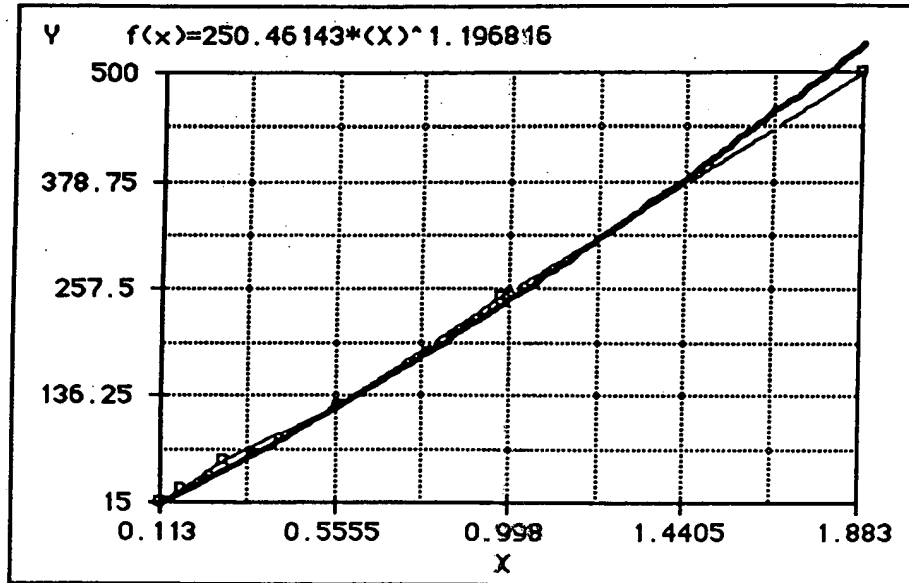
DATA:

.....X.....Y
0.113	15.625
0.1645	31.25
0.2785	62.5
0.5645	125
0.981	250
1.883	500

* EXPONENTIAL FIT *

$$Y = 250.46143 * (X)^{1.196816}$$

Coef. of Regression= 0.993049 For 6 Data Pairs



INITIALS GSS

SUBJECT • Untitled

DATE 2/2/95



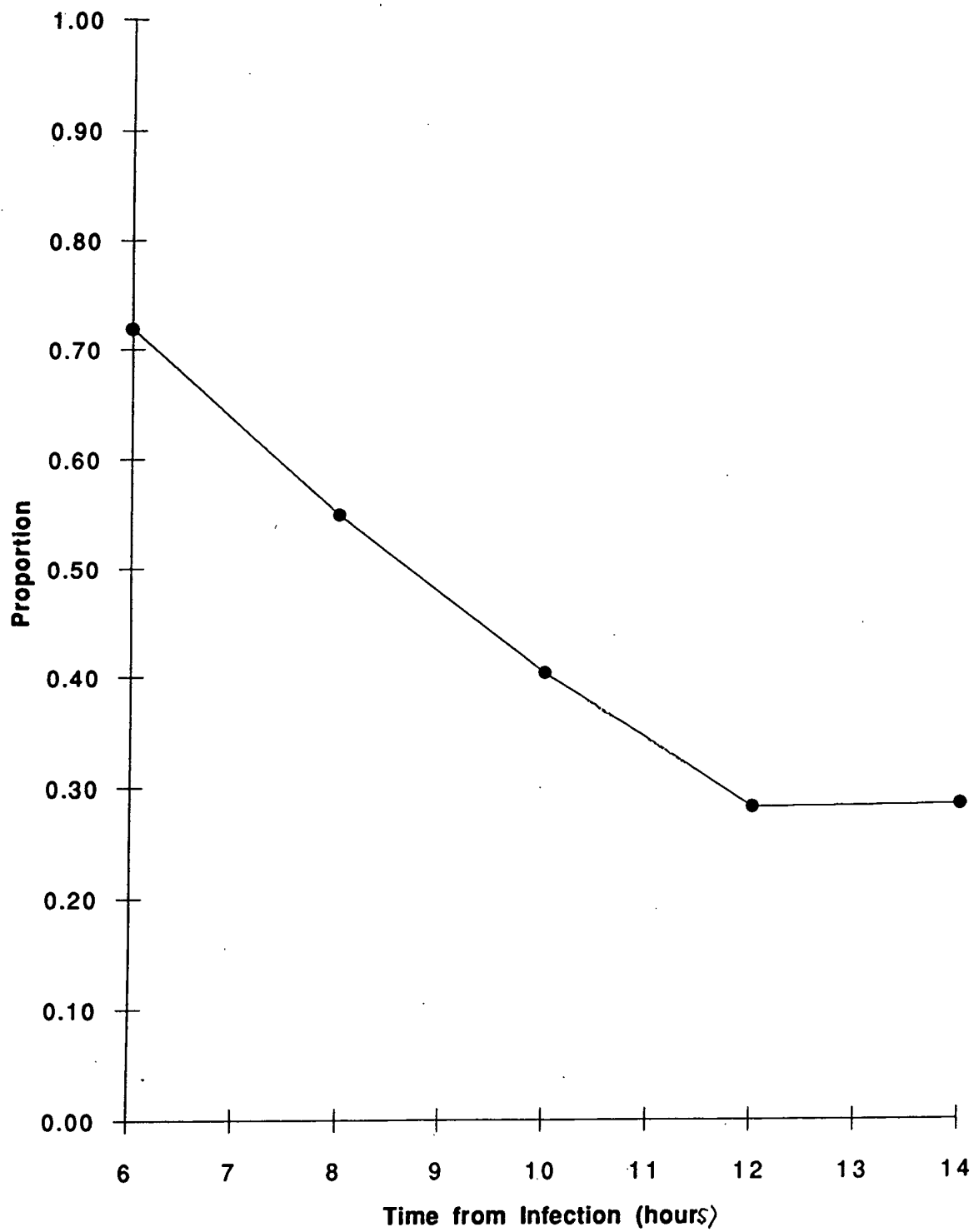
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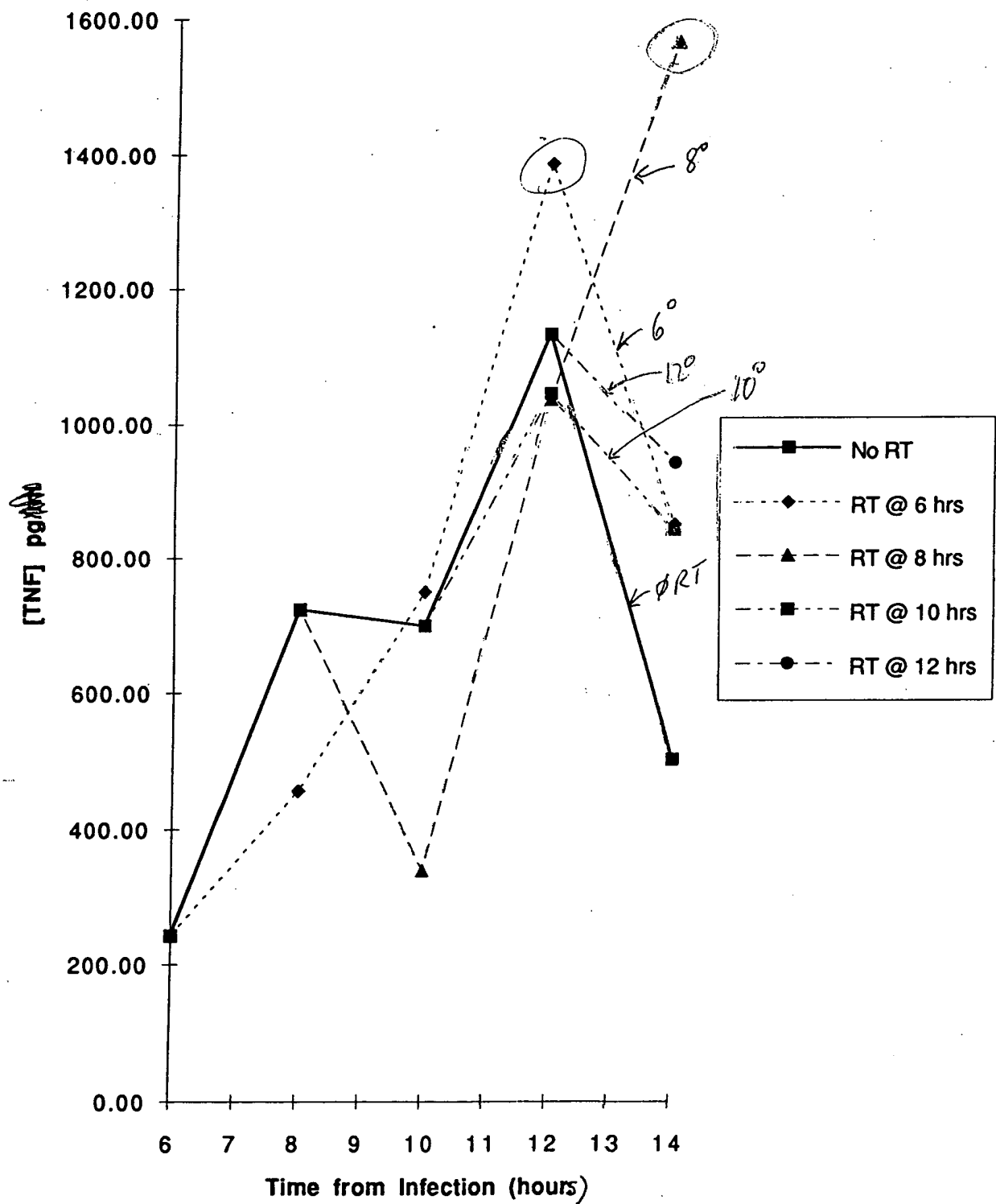
TNF ELISA 2/2/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Total [TNF]
0.107	0.105	0.106	17.07	None	6 hours	supernatant	68.28	243.08
0.225	0.24	0.2325	43.70	None	6 hours	pellet	174.80	
0.389	0.395	0.392	81.66	None	8 hours	supernatant	326.62	723.19
0.46	0.462	0.461	99.14	None	8 hours	pellet	396.57	
0.294	0.299	0.2965	58.46	6 hours	8 hours	supernatant	233.84	456.40
0.281	0.288	0.2845	55.64	6 hours	8 hours	pellet	222.56	
0.484	0.478	0.481	104.31	None	10 hours	supernatant	417.24	699.51
0.349	0.345	0.347	70.57	None	10 hours	pellet	282.27	
0.267	0.262	0.2645	50.99	8 hours	10 hours	supernatant	203.97	339.52
0.188	0.188	0.188	33.89	8 hours	10 hours	pellet	135.55	
0.514	0.534	0.524	115.57	6 hours	10 hours	supernatant	462.27	749.90
0.339	0.366	0.3525	71.91	6 hours	10 hours	pellet	287.63	
0.817	0.865	0.841	203.58	None	12 hours	supernatant	814.32	1134.47
0.379	0.392	0.3855	80.04	None	12 hours	pellet	320.15	
0.755	0.767	0.761	180.63	10 hours	12 hours	supernatant	722.50	1046.13
0.388	0.39	0.389	80.91	10 hours	12 hours	pellet	323.63	
0.709	0.795	0.752	178.07	8 hours	12 hours	supernatant	712.29	1038.41
0.374	0.409	0.3915	81.53	8 hours	12 hours	pellet	326.12	
0.974	1.067	1.0205	256.62	6 hours	12 hours	supernatant	1026.47	1387.28
0.422	0.43	0.426	90.20	6 hours	12 hours	pellet	360.81	
0.431	0.419	0.425	89.95	None	14 hours	supernatant	359.79	503.15
0.196	0.198	0.197	35.84	None	14 hours	pellet	143.36	
0.72	0.737	0.7285	171.43	12 hours	14 hours	supernatant	685.73	943.84
0.316	0.328	0.322	64.53	12 hours	14 hours	pellet	258.11	
0.639	0.655	0.647	148.74	10 hours	14 hours	supernatant	594.96	843.50
0.308	0.316	0.312	62.14	10 hours	14 hours	pellet	248.54	
1.174	1.202	1.188	307.81	8 hours	14 hours	supernatant	1231.23	1566.84
0.399	0.403	0.401	83.90	8 hours	14 hours	pellet	335.62	
0.68	0.661	0.6705	155.23	6 hours	14 hours	supernatant	620.92	852.40
0.284	0.304	0.294	57.87	6 hours	14 hours	pellet	231.48	

Percentage of TNF in "Pellet"



TNF production by interval to RT





THE UNIVERSITY OF CHICAGO
DEPARTMENT OF RADIATION & CELLULAR ONCOLOGY
DIVISION OF THE BIOLOGICAL SCIENCES AND
THE PRITZKER SCHOOL OF MEDICINE

Main Office: (312) 702-6819
Appointment Desk: (312) 702-6860
Facsimile: (312) 702-0610

University of Chicago Medical Center
5841 South Maryland Avenue, MC 0085
Chicago, Illinois 60637

Date: 2/6/95

To: James Linsley
From: *Gregory S. Sibley, MD*
Helena J. Mauceri

J-013.

Please transfer the cages listed below from CLSC 1053 to Carlson room J-019.
Please have the transfer completed by 5:00 p.m. 2/7/95
If this is not possible please call me at 2-0492.

Thank you.

Greg Sibley BP #3439
Cage numbers: EXT 2-0294

~~1859~~
~~AA108958 - AA108964~~ (7 cages)
AA10966 - AA108972
MOUSE # 1859 - 1886

TNF U87 in vitro

2/6/95

PURPOSE - To determine whether TNF production is
 induced with 9 Gy RT following R899-6
 infection (10^6 PFU = \sim MOI = 0.5) in U87 cells.

METHODS - From previous experiments the optimal
 interval from infection to RT was 6 hrs. ~~And~~
 optimal interval following RT was 6-8 hrs
 (total interval 12-14 hrs from infection.) ..

- ① See methods from 2/1/95. Briefly, 10^6 PFU of
 R899-6 was placed in 60 mm plates of
 subconfluent ($\sim 2 \times 10^6$ cells) U87 cells & incubate
 x 2° in 15% media @ 37°.
- ② 10% FCS media + IgG added @ 2°
- ③ 9 Gy RT given @ 6° to RT group.
- ④ Plates sampled at 12 & 14° for "supernatant" & "pellet"

EFFICIENCY LINE® 22-206



	1	2	3	4	5	6	7	8	9
1	1-2	3-4	5-6	7-8	9-10	11-12	13-14		
2	0	12 S	12 P	12 RTS	14 S	14 P	"		
3	15	12 S	12 P	12 RTS	"	"	"		
4	31	12 S	12 P	12 RTP	"	"	14 RTP		
5	63	12 S	12 P	12 RTP	"	"	"		
6	125	12 S	12 RTS	12 RTP	"	14 RTS	"		
7	250	12 S	12 RTS	12 RTP	"	"	"		
8	500	12 P	12 RTS	12 RTP	14 P	"	"		
9	1000	12 P	12 RTS	12 RTP	"	"	"		
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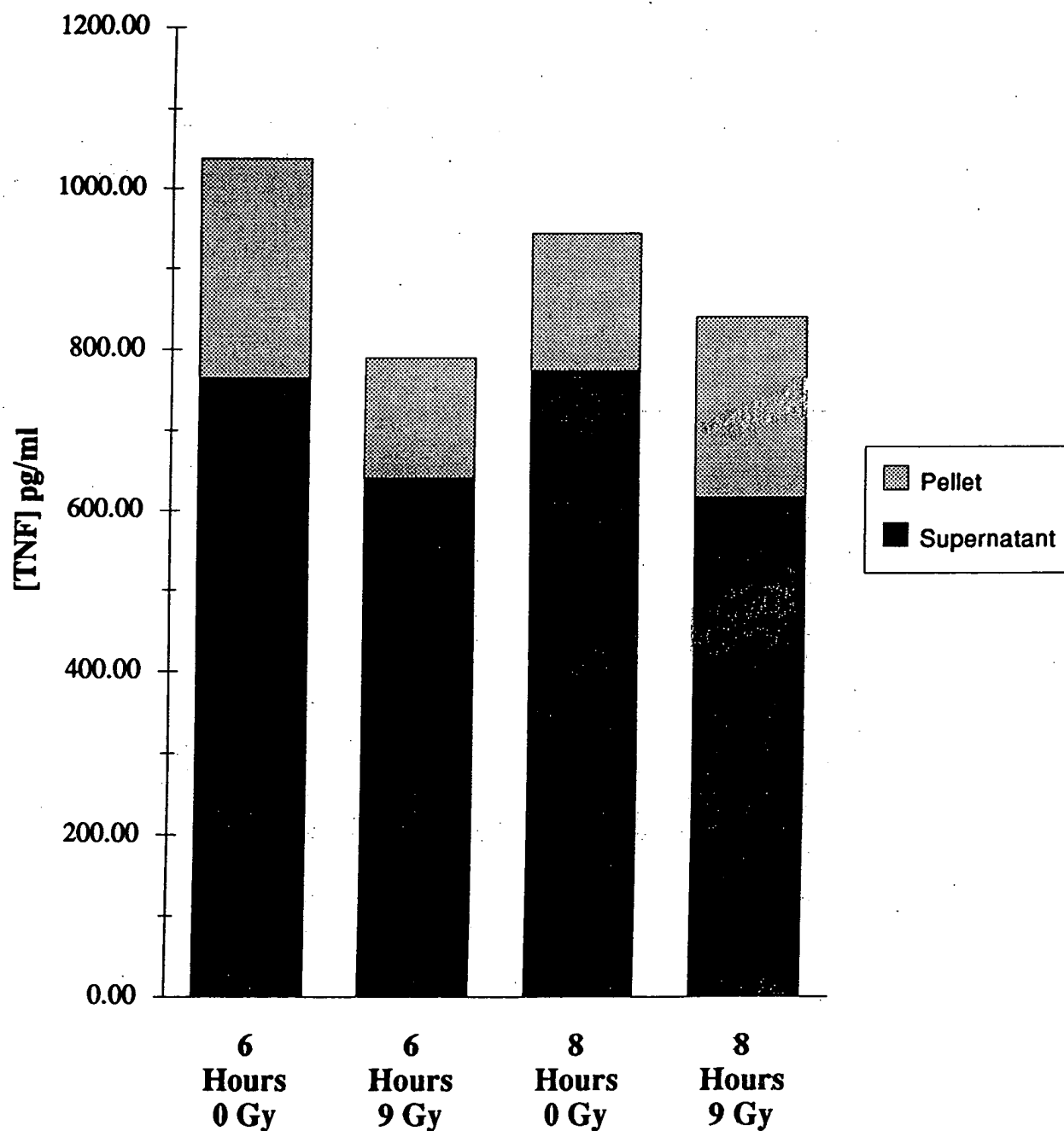
314
112
224

TNF ELISA 2/2/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Ave [TNF]	Total TNF
0.908	0.896	0.902	192.44	none	12 hours	supernatant	769.76	764.69	1038.11
0.846	0.748	0.797	166.82	none	12 hours	supernatant	667.26		
0.892	0.847	0.8695	184.46	none	12 hours	supernatant	737.83		
0.857	0.842	0.8495	179.57	none	12 hours	supernatant	718.27		
0.936	0.945	0.9405	201.96	none	12 hours	supernatant	807.82		
1.014	1.026	1.02	221.79	none	12 hours	supernatant	887.17		
0.368	0.372	0.37	68.78	none	12 hours	pellet	275.11	273.43	
0.383	0.386	0.3845	71.90	none	12 hours	pellet	287.60		
0.363	0.36	0.3615	66.96	none	12 hours	pellet	267.83		
0.392	0.377	0.3845	71.90	none	12 hours	pellet	287.60		
0.366	0.33	0.348	64.08	none	12 hours	pellet	256.31		
0.368	0.351	0.3595	66.53	none	12 hours	pellet	266.12		
0.824	0.884	0.854	180.67	6 hours	12 hours	supernatant	722.66	640.45	789.94
0.791	0.835	0.813	170.69	6 hours	12 hours	supernatant	682.76		
0.833	0.821	0.827	174.09	6 hours	12 hours	supernatant	696.35		
0.734	0.721	0.7275	150.14	6 hours	12 hours	supernatant	600.54		
0.693	0.8	0.7465	154.67	6 hours	12 hours	supernatant	618.69		
0.602	0.686	0.644	130.42	6 hours	12 hours	supernatant	521.69		
0.234	0.251	0.2425	42.23	6 hours	12 hours	pellet	168.91	149.49	
0.188	0.219	0.2035	34.49	6 hours	12 hours	pellet	137.95		
0.21	0.241	0.2255	38.83	6 hours	12 hours	pellet	155.31		
0.234	0.222	0.228	39.33	6 hours	12 hours	pellet	157.30		
0.209	0.21	0.2095	35.66	6 hours	12 hours	pellet	142.66		
0.203	0.196	0.1995	33.71	6 hours	12 hours	pellet	134.82		
0.876	0.93	0.903	192.69	none	14 hours	supernatant	770.75	774.25	943.63
0.813	0.792	0.8025	168.15	none	14 hours	supernatant	672.58		
0.935	0.947	0.941	202.08	none	14 hours	supernatant	808.32		
0.924	0.9	0.912	194.91	none	14 hours	supernatant	779.63		
0.969	0.953	0.961	207.05	none	14 hours	supernatant	828.19		
0.924	0.913	0.9185	196.51	none	14 hours	supernatant	786.04		
0.248	0.249	0.2485	43.44	none	14 hours	pellet	173.74	169.38	
0.27	0.271	0.2705	47.90	none	14 hours	pellet	191.62		
0.251	0.254	0.2525	44.24	none	14 hours	pellet	176.97		
0.233	0.24	0.2365	41.02	none	14 hours	pellet	164.09		
0.244	0.224	0.234	40.52	none	14 hours	pellet	162.09		
0.211	0.221	0.216	36.95	none	14 hours	pellet	147.78		
0.65	0.653	0.6515	132.18	6 hours	14 hours	supernatant	528.71	616.03	840.68
0.722	0.696	0.709	145.74	6 hours	14 hours	supernatant	582.95		
0.62	0.555	0.5875	117.30	6 hours	14 hours	supernatant	469.21		
0.775	0.784	0.7795	162.59	6 hours	14 hours	supernatant	650.38		

0.886	0.814	0.85	179.69	6 hours	14 hours	supernatant	718.76		
0.895	0.861	0.878	186.54	6 hours	14 hours	supernatant	746.16		
0.314	0.294	0.304	54.82	6 hours	14 hours	pellet	219.27	224.66	
0.307	0.303	0.305	55.03	6 hours	14 hours	pellet	220.11		
0.296	0.31	0.303	54.61	6 hours	14 hours	pellet	218.44		
0.295	0.307	0.301	54.19	6 hours	14 hours	pellet	216.78		
0.315	0.312	0.3135	56.80	6 hours	14 hours	pellet	227.20		
0.343	0.329	0.336	61.53	6 hours	14 hours	pellet	246.14		

TNF Production By RT in U87 Cells Following R899-6 Infection



Titers 2/14/95

New Samples (2/13/95) = Virus used for all in vivo exper.

Virus	Dilution	Count	Tm	Titer
3616	10^7	Tm	Tm	
	10^8	23	28	2.6×10^9
899-6	10^7	Tm	Tm	
	10^8	29	47	3.8×10^9

Old samples (used for in vivo exper.)

Virus	Dilution	Count	Tm	Titer
3616	10^7	Tm	Tm	
	10^8	45	36	4.1×10^9
899-6	10^7	Tm	Tm	
	10^8	35	26	3.1×10^9

Greg's Mouse Log

Greg's Mouse Log									
Mouse #	Cage #	Group	21	21	11	Tumvol	weight	Tumvol	weight
1704	AA104550	Control	21	21	11	0	21.7	0	
1717	AA104550	Control	13	12.5	10.5	0		0	
1719	AA104550	Control	13	11.5	6	0		0	
1722	AA104550	Control	14	13.5	10	0		0	
1867	AA108970	Control	-10	8	6				
1868	AA108970	Control	-10	9.5	7.5				
1869	AA108970	Control	-10	9	7				
1870	AA108970	Control	-10.5	8.5	8				
		Control	Mean:			0		Mean:	0
			Std Error:			0		Std Error:	0
			Day:			17		Day:	0
1720	AA105283	10^7 899-6	12	11	7.5	0			
1710	AA105283	10^7 899-6	16	13.5	7	0			0
1707	AA105283	10^7 899-6	16	15	11	0	17.2		0
1713	AA105283	10^7 899-6	6.5	5.5	3	0			0
		10^7 899-6	Mean:			0		Mean:	0
			Std Error:			0		Std Error:	0
			Day:			17		Day:	0
1697	AA104549	10^7 3616	-10	9	7	0			0
1708	AA104549	10^7 3616	8	8.5	5	0			0
1709	AA104549	10^7 3616	9	10	7	0			0
1723	AA104549	10^7 3616	7.5	9	6	0			0
		10^7 3616	Mean:			0		Mean:	0
			Std Error:			0		Std Error:	0
			Day:			17		Day:	0
1859	AA108972	RT Alone	9.5	9.5	5.5	0			0
1860	AA108972	RT Alone	12	10.5	8	0			0
1861	AA108972	RT Alone	10.5	9	7	0			0
1862	AA108972	RT Alone	12	9	7	0			0
		RT Alone	Mean:			0		Mean:	0
			Std Error:			0		Std Error:	0
			Day:			17		Day:	0

[illegible]

[illegible]

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 18781

ARC USE ONLY

PO. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASISREQUEST BY: Greg Sibley, MD DATE: 2/16/95REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: _____

FAS ACCOUNT: 2-7371-5100VENDOR: FCR1REQUESTED DELIVERY DATE: 2/21/95PI: HALLANTANPROTOCOL: 58671PHONE: 2-0294SPECIES: MouseQUANTITY: 60STRAIN: Athymic NudeSEX: M F EITHERWEIGHT/AGE: 25-6 wks ALTERNATE WEIGHT/AGE: _____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER ☒ CLSC _____ FMI _____ OTHER _____



The University of Chicago
Departmental Purchase Order

USA Orders - Cust # 112486
Life Technologies, Inc
P.O. Box 68
Grand Island
NY 14072
State Zip Code
Payment Terms Delivery charge? ☐ Yes ☒ No
Telephone No. 1-800-828-6686 FAX No. 1-800-331-2286

THE UNIVERSITY OF CHICAGO

Dept. Code:

Radiation Oncology MC-0083

Department: Grace Sibley, MD

Room: G-03

5830 S. Ellis Ave

Chicago

City State Zip Code 114 60637

Order placed by phone? ☐ No ☐ Yes

Order placed with (name) Melissa Ref # 372369 2/17/95

Order placed by (name) Grace Sibley, MD

Phone #

Date

Account Code

Payroll No.

Ext.

Date

Authorized Signature

Print Name

Date

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animal; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services that would be obtained from campus departments as stated in University policies & procedures; chaining, etc. are used to exceed the restriction of \$500.00 for one purchase; travel, meals, lodging; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.

2. Order MUST be typewritten.

3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.

4. Vendor and Ship To must have complete addresses.

5. Department Code MUST be filled in with 4-digit department code from listing already furnished.

6. Only ONE account code is allowed per order.

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Purchase Order Number

Z873342

THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

Greg

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
24		11095-023 Minimal Essential Media 500 ml	8.64	207.36
12		15140-015 Penicillin - Streptomycin 100 ml	10.00	120.00
18		22900-055 RPMI 1640 Media 500 ml	12.91	154.92
12		22400-055 RPMI 1640 Media 500 ml	12.91	154.92
				482.28

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

2/25/95

Greg's Mouse Log

Mouse #	Cage #	Group	Mean	Std Error	Day	Tumvol	weight	Tumvol	weight
1704	AA104550	Control							
1717	AA104550	Control	18	15.5	14	0		0	
1719	AA104550	Control	12	15	8	0		0	
1722	AA104550	Control	19.5	18	13	0		0	
1867	AA108970	Control	13	16	9.5			0	
1868	AA108970	Control	18	15	13				
1869	AA108970	Control	15.5	16	9				
1870	AA108970	Control	15.5	13	11				
		Control	Mean:	0				Mean:	0
			Std Error:	0				Std Error:	0
			Day:	17				Day:	0
1720	AA105283	10^7 899-6	11.5	11.5	8	0			
1710	AA105283	10^7 899-6	19.5	16	8	0			
1707	AA105283	10^7 899-6	19	16.5	13	0			
1713	AA105283	10^7 899-6	0			0			
		10^7 899-6	Mean:	0				Mean:	0
			Std Error:	0				Std Error:	0
			Day:	17				Day:	0
1697	AA104549	10^7 3616	11	6.5	5.5	0			
1708	AA104549	10^7 3616	5.5	7	3.5	0			
1709	AA104549	10^7 3616	6	7.5	4	0			
1723	AA104549	10^7 3616	8	9	5	0			
		10^7 3616	Mean:	0				Mean:	0
			Std Error:	0				Std Error:	0
			Day:	17				Day:	0
1859	AA108972	RT Alone	10	11.5	6.5	0			
1860	AA108972	RT Alone	9	8	6.5	0			
1861	AA108972	RT Alone	11.5	12.5	8	0			
1862	AA108972	RT Alone	11	9	4	0			
		RT Alone	Mean:	0				Mean:	0
			Std Error:	0				Std Error:	0
			Day:	17				Day:	0

March 8, 1995

Jim Linsley
P-110
Animal Resources

Dear Jim,

Please transfer the cages listed below from CLSC 1053 to Carlson room J-O13.
Please have the transfer completed by 5 pm on 3/10/95.

If this is not possible, please contact me.

Thank you,

Greg Sibley, M.D.
Dept. of Radiation and Cellular Oncology
Beeper #3439
Extension 2-0294

Cage Numbers:
AA111326 through AA111333

Total Number of Cages: 8

3/9/95

Greg's Mouse Log

Mouse #	Cage #	Group	Turnvol	weight	Turnvol	weight
1720	AA105283	10^7 899-6	+17.5	12	9.5	
1710	AA105283	10^7 899-6	+6.5	6.5	1.5	
1713	AA105283	10^7 899-6	+0	0	0	
		10^7 899-6				
1697	AA104549	10^7 3616	+15.5	13.5	9	
1708	AA104549	10^7 3616	+2	2.5	1.5	
1709	AA104549	10^7 3616	+5	5	1	
1723	AA104549	10^7 3616	+0	0	0	
1875	AA108968	10^7 3616	+19	18.5	10	
1876	AA108968	10^7 3616	+18	17	11.5	
1877	AA108968	10^7 3616	+18.5	14	13	
1878	AA108968	10^7 3616	+16	12.5	11	
		10^7 3616				
1859	AA108972	RT Alone	9	1.5	4	
1860	AA108972	RT Alone 13x11x6	8.5	7.5	5	
1861	AA108972	RT Alone	10	10.5	7	
1862	AA108972	RT Alone	8.5	7.5	3	
1891	AA108964	RT Alone	+6.5	6.5	5	
1892	AA108964	RT Alone	+12.5	9.5	6	
1893	AA108964	RT Alone	+10	9.5	5.5	
1894	AA108964	RT Alone	+8	8	5	
		RT Alone				
1863	AA108971	10^7 3616+RT	+10	8	4	
1864	AA108971	10^7 3616+RT	+10.5	7	4	
1865	AA108971	10^7 3616+RT	+5.5	6.5	4.5	
1866	AA108971	10^7 3616+RT	+7	5	4.5	
1883	AA108966	10^7 3616+RT	+3.5	3	1.5	
1884	AA108966	10^7 3616+RT	+8	8	6	
1885	AA108966	10^7 3616+RT	+9	8.5	4	
1886	AA108966	10^7 3616+RT	+3.5	4	2	
		10^7 3616+RT				

1871	AA108969	10^7 899-6+RT	7.5	7	4.5
1872	AA108969	10^7 899-6+RT	7	5.5	4.5
1873	AA108969	10^7 899-6+RT	8	7.5	4.5
1874	AA108969	10^7 899-6+RT	8	7.5	4
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	3.5	3	1
1881	AA108967	10^7 899-6+RT	5.5	6	3
1882	AA108967	10^7 899-6+RT	4	3	1
		10^7 899-6+RT			
2032	AA111326	10^7 899-6			
2033	AA111326	10^7 899-6			
2034	AA111326	10^7 899-6			
2035	AA111326	10^7 899-6			
2036	AA111327	10^7 899-6			
2037	AA111327	10^7 899-6			
2038	AA111327	10^7 899-6			
2039	AA111327	10^7 899-6			
2040	AA111328	10^7 899-6			
2041	AA111328	10^7 899-6			
2042	AA111328	10^7 899-6			
2043	AA111328	10^7 899-6			
		10^7 899-6			
2044	AA111329	10^7 3616			
2045	AA111329	10^7 3616			
2046	AA111329	10^7 3616			
2047	AA111329	10^7 3616			
2048	AA111330	10^7 3616			
2049	AA111330	10^7 3616			
2050	AA111330	10^7 3616			
2051	AA111330	10^7 3616			
		10^7 3616			
2052	AA111331	10^7 899-6+RT			
2053	AA111331	10^7 899-6+RT			
2054	AA111331	10^7 899-6+RT			
2055	AA111331	10^7 899-6+RT			
2056	AA111332	10^7 899-6+P			
2057	AA111332	10^7 899-6			
2058	AA111332	10^7 899-6			
2059	AA111332	10^7 899-6			
		10^7 899-6+h			

2060	AA111333	10^7 3616+RT
2061	AA111333	10^7 3616+RT
2062	AA111333	10^7 3616+RT
2063	AA111333	10^7 3616+RT
2067	AA111335	10^7 3616+RT
2068	AA111335	10^7 3616+RT
2069	AA111335	10^7 3616+RT
2070	AA111335	10^7 3616+RT
		10^7 3616+RT

3/13/95

Greg's Mouse Log

Mouse #	Cage #	Group	Tumvol	weight	Tumvol	weight
1720	AA105283	10^7 899-6				
1710	AA105283	10^7 899-6				
1713	AA105283	10^7 899-6				
		10^7 899-6				
1697	AA104549	10^7 3616				
1708	AA104549	10^7 3616				
1709	AA104549	10^7 3616				
1723	AA104549	10^7 3616				
1875	AA108968	10^7 3616				
1876	AA108968	10^7 3616				
1877	AA108968	10^7 3616				
1878	AA108968	10^7 3616				
		10^7 3616				
1859	AA108972	RT Alone				
1860	AA108972	RT Alone				
1861	AA108972	RT Alone				
1862	AA108972	RT Alone				
1891	AA108964	RT Alone				
1892	AA108964	RT Alone				
1893	AA108964	RT Alone				
1894	AA108964	RT Alone				
		RT Alone				
1863	AA108971	10^7 3616+RT				
1864	AA108971	10^7 3616+RT				
1865	AA108971	10^7 3616+RT				
1866	AA108971	10^7 3616+RT				
1883	AA108966	10^7 3616+RT				
1884	AA108966	10^7 3616+RT				
1885	AA108966	10^7 3616+RT				
1886	AA108966	10^7 3616+RT				
		10^7 3616+RT				

3/13/95

1871	AA108969	10^7 899-6+RT											
1872	AA108969	10^7 899-6+RT											
1873	AA108969	10^7 899-6+RT											
1874	AA108969	10^7 899-6+RT											
1879	AA108967	10^7 899-6+RT											
1880	AA108967	10^7 899-6+RT											
1881	AA108967	10^7 899-6+RT											
1882	AA108967	10^7 899-6+RT											
		10^7 899-6+RT											
2032	AA111326	10^7 899-6 WRT	9	7	6		22.2	3616	+RT				
2033	AA111326	10^7 899-6 WRT	11	7.5	6.5		22.6	"					
2034	AA111326	10^7 899-6 WRT	11	10	7		19.6	"					
2035	AA111326	10^7 899-6 WRT	12.5	7	6		19.0	"					
2036	AA111327	10^7 899-6 WRT	10	7	5		19.7	3616	+RT				
2037	AA111327	10^7 899-6 WRT	8	7	4.5		21.7	"					
2038	AA111327	10^7 899-6 WRT	11.5	9.5	7.5		13.0	"					
2039	AA111327	10^7 899-6 WRT	9.5	7	5		15.5	"					
2040	AA111328	10^7 899-6	11	9.5	7		21.3	3616					
2041	AA111328	10^7 899-6	6.5	6	5		21.3	899-6+RT	11				
2042	AA111328	10^7 899-6	10	10	7.5		23.2	"					
2043	AA111328	10^7 899-6	8	6.5	5.5		23.7	"					
		10^7 899-6											
2044	AA111329	10^7 3616+RT	8.5	7	4.5		19.3	899-6	+RT				
2045	AA111329	10^7 3616+RT	7	6	4		22.4	"					
2046	AA111329	10^7 3616 "	6.5	6	5		20.7	"					
2047	AA111329	10^7 3616 "	9	8.5	5		21.5	"					
2048	AA111330	10^7 3616+RT	8	5	4.5		19.2	899-6	+RT				
2049	AA111330	10^7 3616 "	11	7	5.5		22.6	"					
2050	AA111330	10^7 3616 "	8.5	6.5	5.5		21.1	"					
2051	AA111330	10^7 3616 "	8.5	8.5	5.5		19.2	"					
		10^7 3616											
2052	AA111331	10^7 899-6+RT	7	6	5		22.4	899-6					
2053	AA111331	10^7 899-6+RT	7	6	4.5		22.7	899-6					
2054	AA111331	10^7 899-6+RT	7	6	4.5		20.1	"					
2055	AA111331	10^7 899-6+RT	"	6.5	5		18.3	"					
2056	AA111332	10^7 899-6+RT	8	5	4		21.7	899-6					
2057	AA111332	10^7 899-6+RT	7	6	4.5		21.8	"					
2058	AA111332	10^7 899-6+RT	7	9	5.5		22.9	"					
2059	AA111332	10^7 899-6+RT						"					
		10^7 899-6+RT											

extra injected

Bubba tumors

1331

1 tumor

1332

2 tumors

899-6 899-6 899-6

2060	AA111333	10^7 3616+RT
2061	AA111333	10^7 3616+RT
2062	AA111333	10^7 3616+RT
2063	AA111333	10^7 3616+RT
2067	AA111335	10^7 3616+RT
2068	AA111335	10^7 3616+RT
2069	AA111335	10^7 3616+RT
2070	AA111335	10^7 3616+RT
		10^7 3616+RT

Greg's Mouse Log

Greg's Mouse Log									
Mouse #	Cage #	Group	3/13/95	3/13/95	3/13/95	Tumvol	weight		
1720	AA105283	10^7 899-6	-20.5	10	13	26.0			
1710	AA105283	10^7 899-6	-0	0	0	23.0			
1713	AA105283	10^7 899-6	-0	0	0	21.6			
		10^7 899-6							
1697	AA104549	10^7 3616	-17.5	14.5	11	23.8			
1708	AA104549	10^7 3616	-0	0	0	24.0			
1709	AA104549	10^7 3616	-3.5	4	1	28.2			
1723	AA104549	10^7 3616	-0	0	0	26.3			
1875	AA108968	10^7 3616	-19	20	10	22.3			
1876	AA108968	10^7 3616	-19	17	13.5	23.5			
1877	AA108968	10^7 3616	-22.5	17.5	16.5	27.3			
1878	AA108968	10^7 3616	-18.5	15.5	15.5	26.0			
		10^7 3616							
1859	AA108972	RT Alone	10	10	5	25.1			
1860	AA108972	RT Alone	13	1	8.5	8.7			
1861	AA108972	RT Alone	11	1.5	7	20.9			
1862	AA108972	RT Alone	9	7.5	3.5	24.7			
1891	AA108964	RT Alone	6.5	7.5	3.5	19.8			
1892	AA108964	RT Alone	12.5	9.5	5	21.5			
1893	AA108964	RT Alone	10	9	5.5	20.8			
1894	AA108964	RT Alone	7.5	8	4	21.5			
		RT Alone							
1863	AA108971	10^7 3616+RT	-9.5	8	3.5	22.9			
1864	AA108971	10^7 3616+RT	-6	5	4	25.3			
1865	AA108971	10^7 3616+RT	-10	6.5	4	24.9			
1866	AA108971	10^7 3616+RT	-5	4	3.5	25.1			
1883	AA108966	10^7 3616+RT	-4	3	1	21.7			
1884	AA108966	10^7 3616+RT	-8.5	7	4.5	23.5			
1885	AA108966	10^7 3616+RT	Dead	3/13/95					
1886	AA108966	10^7 3616+RT	-3	4	2	22.5			
		10^7 3616+RT							

1871	AA108969	10^7 899-6+RT	- 8	7	5	24.3
1872	AA108969	10^7 899-6+RT	- 12	5.5	4	22.5
1873	AA108969	10^7 899-6+RT	- 8	7.5	4.5	23.3
1874	AA108969	10^7 899-6+RT	- 8	8	4	26.1
1879	AA108967	10^7 899-6+RT	- 0	0	0	25.0
1880	AA108967	10^7 899-6+RT	- 0	0	0	21.3
1881	AA108967	10^7 899-6+RT	- 5.5	5.5	2.5	29.0
1882	AA108967	10^7 899-6+RT	- 4	3	1	22.5
		10^7 899-6+RT				
2032	AA111326	10^7 899-6				
2033	AA111326	10^7 899-6				
2034	AA111326	10^7 899-6				
2035	AA111326	10^7 899-6				
2036	AA111327	10^7 899-6				
2037	AA111327	10^7 899-6				
2038	AA111327	10^7 899-6				
2039	AA111327	10^7 899-6				
2040	AA111328	10^7 899-6				
2041	AA111328	10^7 899-6				
2042	AA111328	10^7 899-6				
2043	AA111328	10^7 899-6				
		10^7 899-6				
2044	AA111329	10^7 3616				
2045	AA111329	10^7 3616				
2046	AA111329	10^7 3616				
2047	AA111329	10^7 3616				
2048	AA111330	10^7 3616				
2049	AA111330	10^7 3616				
2050	AA111330	10^7 3616				
2051	AA111330	10^7 3616				
		10^7 3616				
2052	AA111331	10^7 899-6+RT				
2053	AA111331	10^7 899-6+RT				
2054	AA111331	10^7 899-6+RT				
2055	AA111331	10^7 899-6+RT				
2056	AA111332	10^7 899-6+RT				
2057	AA111332	10^7 899-6+RT				
	AA111332	10^7 899-6+RT				
	AA111332	10^7 899-6+RT				
		10^7 899-6+RT				

[illegible]

1871	AA108969	10^7 899-6+RT	6	6	3.5
1872	AA108969	10^7 899-6+RT	5.5	5	3
1873	AA108969	10^7 899-6+RT	7	7	3.5
1874	AA108969	10^7 899-6+RT	7.5	7.5	3
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	0	0	0
1881	AA108967	10^7 899-6+RT	5.8	6	2.5
1882	AA108967	10^7 899-6+RT	3	2.5	1
		10^7 899-6+RT			
2032	AA111326	10^7 899-6	11	8	6
2033	AA111326	10^7 899-6	13	9	6
2034	AA111326	10^7 899-6	11.5	11	7
2035	AA111326	10^7 899-6	12.5	8.5	8
2036	AA111327	10^7 899-6	11.5	9.5	6
2037	AA111327	10^7 899-6	9.5	8	5.5
2038	AA111327	10^7 899-6	13	11	8.5
2039	AA111327	10^7 899-6	10.5	8.5	6
2040	AA111328	10^7 899-6	13	13	8
2041	AA111328	10^7 899-6	8	7.5	5.5
2042	AA111328	10^7 899-6	12	14	11
2043	AA111328	10^7 899-6	11	8.5	6
		10^7 899-6			
2044	AA111329	10^7 3616	11	9	7
2045	AA111329	10^7 3616	8	6.5	4.5
2046	AA111329	10^7 3616	9	7.5	6
2047	AA111329	10^7 3616	10.5	10.5	5
2048	AA111330	10^7 3616	9	6	4.5
2049	AA111330	10^7 3616	15	8	6.5
2050	AA111330	10^7 3616	9	7.5	5.5
2051	AA111330	10^7 3616	11	10	5.5
		10^7 3616			
2052	AA111331	10^7 899-6+RT	10	7.5	6.5
2053	AA111331	10^7 899-6+RT	6.5	8	6
2054	AA111331	10^7 899-6+RT	9.5	7.5	4.5
2055	AA111331	10^7 899-6+RT	14	7.5	6.5
2056	AA111332	10^7 899-6+RT	10	7	4.5
2057	AA111332	10^7 899-6+RT	7.5	7	5
2058	AA111332	10^7 899-6+RT	10	12	5
2059	AA111332	10^7 899-6+RT			
		10^7 899-6+RT			

[illegible]

3/20

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0	weight	Day 3	weight
1720	AA105283	10^7 899-6	23 16 14	25.2		
1710	AA105283	10^7 899-6	0 0 0	23.3		
1713	AA105283	10^7 899-6	0 0 0	22.1		
2052	AA111331	10^7 899-6	11 9.5 6	18.4		
2053	AA111331	10^7 899-6	8 10.5 7	21.1		
2054	AA111331	10^7 899-6	7 6 4.5	19.5		
2055	AA111331	10^7 899-6	10 12 7.5	17.4		
2056	AA111332	10^7 899-6	9 7 5	23.4		
2057	AA111332	10^7 899-6	7.5 6 4.5	21.8		
2058	AA111332	10^7 899-6	11 9.5 5.5	23.5		
2060	AA111333	10^7 899-6	11 8.5 7	22.3		
2061	AA111333	10^7 899-6	10 9 7	23.3		
2062	AA111333	10^7 899-6	9 7.5 6.5	18.0		
2063	AA111333	10^7 899-6	9.5 7.5 5.5	20.5		
		10^7 899-6				
1697	AA104549	10^7 3616	18 15 11.5	23.4		
1708	AA104549	10^7 3616	5 3 1	22.5		
1709	AA104549	10^7 3616	3 3 1	27.6		
1723	AA104549	10^7 3616	0 0 0	26.1		
1875	AA108968	10^7 3616	20.5 16.5 9	18.3	→ 52 L	
1876	AA108968	10^7 3616	Dead			
1877	AA108968	10^7 3616	26 19 13	26.3		
1878	AA108968	10^7 3616	22 13.5 17	23.8		
2040	AA111328	10^7 3616	16 14 8.5	22.2	→ AA 111 335	
2041	AA111328	10^7 3616	6 5.5 4	22.2		
2042	AA111328	10^7 3616	17 17 12	23.6	→ 11	
2043	AA111328	10^7 3616	10.5 9 5	24.0		
2064	AA111334	10^7 3616	9.5 7 7	21.4		
2065	AA111334	10^7 3616	8.5 8.5 10	21.1		
2066	AA111334	10^7 3616	11 7.5 6	16.5		
2067	AA111334	10^7 3616	11 9.5 8	22.2		
		10^7 3616				
2070			10.5 7 6	23.3		
1859	AA108972	RT Alone	7-14.5 14 11	18.5		
1860	AA108972	RT Alone	8 10 5.5	24.0		
1861	AA108972	RT Alone	11.5 12 7.5	21.0		
1862	AA108972	RT Alone	8 6.5 3.5	23.5		
1891	AA108964	RT Alone	Dead			
1892	AA108964	RT Alone	8 8 5 under	19.2		
1893	AA108964	RT Alone	Dead			
1894	AA108964	RT Alone	Dead			
		RT Alone				

[illegible]

3/21/95

In vivo TNF induction

PURPOSE - To see if TNF α induction production is induced in vivo with 20 Gy delivered 24 $^{\circ}$ after injection of U87 hind limb tumors with R839-6 (10^7 PFU)

METHODS -

1. Implant subcut tumors in hind limb of nude mice
2. Grow to ~ 100 mm 3 in size
3. Inject with 2×10^7 PFU R839-6 (10 μ l of stock)
4. 24 hours after injection randomize to 4-20 Gy, and sacrifice at the following intervals:

Time	ORT	\oplus RT	
	2 $^{\circ}$ +24 $^{\circ}$	4 mice	4 mice
\rightarrow	6 $^{\circ}$ +24 $^{\circ}$	"	"
\rightarrow	12 $^{\circ}$ +24 $^{\circ}$	"	"
\rightarrow	24 $^{\circ}$ +24 $^{\circ}$	"	"
\rightarrow	48 $^{\circ}$ +24 $^{\circ}$	"	"
\rightarrow	1 WK+24 $^{\circ}$	"	"
\rightarrow	2 WK+24 $^{\circ}$	"	"
	3 WK+24 $^{\circ}$	"	"
			Need 64 mice
			Infect W 9 AM
			RT Th 9 AM
			Sac @ 3 PM
			9 AM
			F 9 AM

IN VIVO TNF INDUCTION

	1	2	3	4	5	6	7	8	9
A	1000		2161	6°	2185	12°	2169	24°	2181
B	500		2162	+ RT	2186	+	2170	+	2182
C	250		2163		2187	RT	2171	2172	2183
D	125		2164		2188		2172		2184
E	62		2177						
F	31°		2178	6°	2141	12°	2165	24°	2173
G	16°		2179	no RT	2142	no	2166	2167	2174
H	0		2180		2143	RT	2167	RT	2175
					2144		2168		2176
	10	11	12	13	14	15	16	17	18
A	48°	2153	1wk	2149	2150	1702	control	2081	control/2
B	+RT	2154	+	<u>2150</u>	2151	1698	control	2145	2°
C		2155	RT	2151	2152	1724	control	2146	2° RT
D		2156		2152		2042	2147	2147	2° RT
E									
F	48°	2157	2WK	2137	2WK	2034	2081	2081	all empty
G	no RT	2158	no	2138	no	2077	899-6	2145	
H		2159	RT	2139	RT	2078	89, 1	2146	
		2160		2140		2080	control 1	2147	
	19								
A	10 ⁷ 6° +RT								
B	10 ⁷ 6° no RT								
C	10 ⁸ 6° +RT								
D	10 ⁸ 6° no RT								
E	10 ⁷ 24° +RT								
F	10 ⁷ 24° no RT								
G	10 ⁸ 24° +RT								
H	10 ⁸ 24° no RT								

not all supernatant assayed
he was spilled

* for these sets, there was insufficient supernatant to fill the second well

INDUCTION-in vivo							
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor Wt	Vol Buffer	[TNF] pg/ml
1702	Control	None	2/16/95	63	0.06	6	0.37
1698	Control	None	2/9/95	67	0.07 2.0	5 1.0	1.03
1724	Control	None	2/16/95	2520	2.5 0.7	6	0.42
2042	3616	3/13/95	4/3/95	528	0.6	6	0.33
2034	3616+20+25	3/13/95	4/3/95	644	0.7	6	0.41
2077	899-6	3/13/95	4/3/95	2090	2.9	1.0	0.47 0.89
2078	899-6	3/13/95	4/3/95	6186	4.5	1.0	0.44
2080	Control 1	None	4/3/95	938	0.9	6	0.52
2081	Control 2	None	4/3/95	1124	1	1.0	1.31
2145	2 hrs + RT	4/26/95	4/27/95		0.51	6	0.57
2146	2 hrs + RT	4/26/95	4/27/95		0.39	6	0.49
2147	2 hrs + RT	4/26/95	4/27/95		0.06	6	0.40

8

52
6
3/2
8
39.2

40ml 150 mM NaCl

400 ml TRIS 1M pH 7.5

400 ml EDTA 0.5M pH 7.5

protease inhibitors

/ DTT 1mM 40ml

/ PMSF 100mM 20ml

leupeptin 5mg 4ml
2 aprotonin 10mg 8ml

26 rows = 39 ml

2/52

76
2
8/152 wells

19 rows
8
152

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT REQUEST

(#315B)

REQ NO 18784

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: _____ SHIPMENTS ON A _____ BASISREQUEST BY: Greg Sibbey, MD DATE: 3/21/95REQUESTORS PHONE NUMBER: 2-0294AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 2-73731-5100 PI: HallgrenVENDOR: FCR1 PROTOCOL: 58671REQUESTED DELIVERY DATE: 3/27/95 PHONE: 2-0294SPECIES: Mouse QUANTITY: 80STRAIN: Albino Nude SEX: M ☒ EITHERWEIGHT/AGE: 5-6 weeks ALTERNATE WEIGHT/AGE: _____
(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)MICROISOLATOR TOP: YES ☒ NO ☐
(ADULTS ONLY)

SPECIAL REQUIREMENTS: _____

PROCUREMENT DESK: 2-9364

HOUSE AT: _____ CARLSON _____ WYLER ☒ CLSC _____ FMI _____ OTHER _____

3/23

1863	AA108971	10^7 3616+RT	10.5	8	3
1864	AA108971	10^7 3616+RT	6	8	3.5
1865	AA108971	10^7 3616+RT	3	3	2
1866	AA108971	10^7 3616+RT	3	3.5	1.5
1883	AA108966	10^7 3616+RT	4.5	4	1
1884	AA108966	10^7 3616+RT	7.5	6	5
1886	AA108966	10^7 3616+RT	6	4	3
2074	AA108966	10^7 3616+RT	6.5	5	7
2032	AA111326	10^7 3616+RT	10	6	5.5
2033	AA111326	10^7 3616+RT	12	9	5.5
2035	AA111326	10^7 3616+RT	8.5	7.5	6
2072	AA111326	10^7 3616+RT	13	9.5	8
2036	AA111327	10^7 3616+RT	12	9	6
2037	AA111327	10^7 3616+RT	8.5	7	6
2039	AA111327	10^7 3616+RT	10	9	5
2073	AA111327	10^7 3616+RT	12.5	11.5	9
		10^7 3616+RT			
1871	AA108969	10^7 899-6+RT	6.5	7	4
1872	AA108969	10^7 899-6+RT	4.5	6.5	2.5
1873	AA108969	10^7 899-6+RT	8	8	3.5
1874	AA108969	10^7 899-6+RT	7	6	3
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	3.5	3	1
1881	AA108967	10^7 899-6+RT	6	4.5	2
1882	AA108967	10^7 899-6+RT	3	2.5	1
2044	AA111329	10^7 899-6+RT	9.5	8	5
2045	AA111329	10^7 899-6+RT	5.5	5	3
2046	AA111329	10^7 899-6+RT	9.5	8	5
2047	AA111329	10^7 899-6+RT	6	6	3.5
2048	AA111330	10^7 899-6+RT	7.5	6	4.5
2049	AA111330	10^7 899-6+RT	15	10	8
2050	AA111330	10^7 899-6+RT	7	6.5	4
2051	AA111330	10^7 899-6+RT	11	13	5
		10^7 899-6+RT			



The University of Chicago Departmental Purchase Order

Z89058

THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

Vendor Name Sarstedt
St James Church Rd
Newton, N.C. 28658
City Newton State NC Zip Code 28658
Payment terms Net 30 Delivery charge? ☐ Yes ☒ No
Telephone No. 800-257-5101 FAX No. 800-257-5101

Ship To THE UNIVERSITY OF CHICAGO
ADDITION ENCLOSURE MC-0085
Greg Sibley G-03
Department CHGO Room 116 Zip Code 60637

Authorized Signature _____ Payroll No. _____
Print Name _____ Ext. _____
Account Code _____ Date _____

Order placed by phone? ☐ No ☒ Yes
Order placed with (name) May Sibley Date 3/24/95
Order placed by (name) Greg Sibley Phone # 800-257-5101

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1000	65816 Time stopper	19.22	
1	1000	55.518 13 ml centrifuge tubes	57.92	
			77.14	
			ORDER TOTAL	

NOT VALID IF TOTAL EXCEEDS \$500.00

BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

DEPARTMENT COPY

Greg's Mouse Log

3/27/94									
Mouse #	Cage #	Group	Day 0			weight	Day 3		
1720	AA105283	10^7 899-6	25	17.5	14	26.5			
1710	AA105283	10^7 899-6	0	0	0	22.7			
2075	AA105283	10^7 899-6	10	9	3	20.7			
1713	AA105283	10^7 899-6	0	0	0	21.2			
2052	AA111331	10^7 899-6	14.5	12	8	20.3			
2053	AA111331	10^7 899-6	12	15.5	8	22.7			
2054	AA111331	10^7 899-6	14	15	6	19.7			
2055	AA111331	10^7 899-6	11	7	3.5	21.9			
2056	AA111332	10^7 899-6	12	15	7	24.5			
2057	AA111332	10^7 899-6	6	6	3	21.4			
2058	AA111332	10^7 899-6	12.5	11	6	23.9			
2076	AA111332	10^7 899-6	9.5	11	7	24.6			
2060	AA111333	10^7 899-6	14	12.5	4.5	25.3	12.5	12.5	8
2061	AA111333	10^7 899-6	11	10	5	22.6			
2062	AA111333	10^7 899-6	8	6	4	16.9			
2063	AA111333	10^7 899-6	9	8	6.5	21.0			
		10^7 899-6							
1697	AA104549	10^7 3616	19	17.5	9.5	25.3			
1708	AA104549	10^7 3616	0	0	0	21.9			
1709	AA104549	10^7 3616	0	0	0	27.3			
1723	AA104549	10^7 3616	0	0	0	26.3			
1877	AA108968	10^7 3616							
1878	AA108968	10^7 3616							
2041	AA111328	10^7 3616	6	5	3	22.3			
2043	AA111328	10^7 3616	13.5	12	7	25			
2064	AA111334	10^7 3616	8	6.5	4	23.1			
2065	AA111334	10^7 3616	16	16	9	23.3			
2066	AA111334	10^7 3616	12	12	6	23.2			
2067	AA111334	10^7 3616	8.5	6	3.5	14.5			
2068		10^7 3616	7	6	5	15.8			
2069		10^7 3616	10	10	7.5	23.2			
2070		10^7 3616	11	8	5	23.4			
2071		10^7 3616	11	10	5	24.0			
		10^7 3616							
1859	AA108972	RT Alone	9.5	10	3.5	23.9			
1860	AA108972	RT Alone	3/27/95						
1861	AA108972	RT Alone	12	13.5	7	21.5			
1862	AA108972	RT Alone	8	7	3	25.3			
1892	AA108964	RT Alone	7.5	8	5	22.8			
		RT Alone							
			12	13.5	7	21.5			

1863	AA108971	10^7 3616+RT	10	7	2.5	22.9
1864	AA108971	10^7 3616+RT	7.5	5	3	24.1
1865	AA108971	10^7 3616+RT	3.5	3	1	24.9
1866	AA108971	10^7 3616+RT	3	3	1	24.9
1883	AA108966	10^7 3616+RT	0	0	0	22
1884	AA108966	10^7 3616+RT	5.5	5.5	3	20.6
1886	AA108966	10^7 3616+RT	11.5	10	7	14.8
2074	AA108966	10^7 3616+RT	4	4	1	22
2032	AA111326	10^7 3616+RT	11.5	7.5	5	20.2
2033	AA111326	10^7 3616+RT	11.5	9	6	21.6
2035	AA111326	10^7 3616+RT	5.5	5.5	3	27.7
2072	AA111326	10^7 3616+RT	13	10	6	18.8
2036	AA111327	10^7 3616+RT	12	9	4	20.4
2037	AA111327	10^7 3616+RT	6	8	4	23.6
2039	AA111327	10^7 3616+RT	11	8	7.5	19.3
2073	AA111327	10^7 3616+RT	8.5	10	5	17.9
		10^7 3616+RT				
1871	AA108969	10^7 899-6+RT	7	7	3.5	23.3
1872	AA108969	10^7 899-6+RT	4.5	6	3	22.4
1873	AA108969	10^7 899-6+RT	7.5	8	4	22.4
1874	AA108969	10^7 899-6+RT	6.5	5	2.5	24.8
1879	AA108967	10^7 899-6+RT	0	0	0	26.0
1880	AA108967	10^7 899-6+RT	0	0	0	20.7
1881	AA108967	10^7 899-6+RT	0	0	0	24.4
1882	AA108967	10^7 899-6+RT	4	3	1	22.1
2044	AA111329	10^7 899-6+RT	9	7.5	5.5	20.9
2045	AA111329	10^7 899-6+RT	7	5	3	24.5
2046	AA111329	10^7 899-6+RT	8.5	7	5	22.7
2047	AA111329	10^7 899-6+RT	5	5	5	21.2
2048	AA111330	10^7 899-6+RT	5.5	8	3.5	19.2
2049	AA111330	10^7 899-6+RT	15	10.5	5	21.8
2050	AA111330	10^7 899-6+RT	6	6	4	21.4
2051	AA111330	10^7 899-6+RT	12.5	11	4.5	20.5
		10^7 899-6+RT				

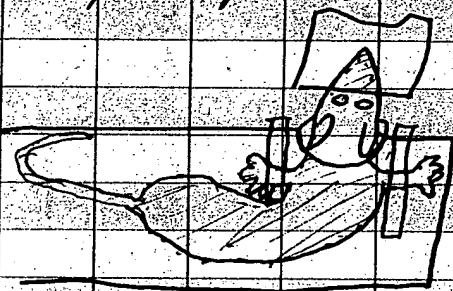
3/27/95

Tolerance of Nude Mice To Whole Brain RT

Purpose - In preparation for stereotactic brain protocol, need to determine maximum tolerated whole brain dose

PROCEDURE -

- ① Anesthetize mice w/ Ketamine/Xylazine @ 4 ml
- ② Tape front legs of mice with lead protecting through lead shield & piece of lead over nose (eyes exposed)



- ③ Irradiate w/ Maxtron: Plexiglass-to-coll dist = 8 cm, 30 mA 50 KVP (see TLD measurements next page)
TLD @ surface of skull = 450-500 cGy
TLD under head = 325 cGy } 500 cGy includes

- ④ Plot experiment = 3 mice
mouse #1 = $1000 \times 5 = 5000$ cGy
#2 = $500 \times 6 = 3000$ cGy
#3 = $20 \times 8 = 4000$ cGy

RESULTS -

	RT dates		status
	start	end	
mouse #1	3/27	3/31	died 4/5
mouse #2	3/27	4/3	
mouse #3	3/27	4/5	

3-27-95 TLD exposure Measured on March 27
 Dr. S. W. H. (Hypox) Channel 0354 71A
 100 KVP 30 mA 2 min exposure to button OK
 1 mm Al filter collimator

Calibration TLDs

500 cGy at skin beam quality

11.34 mC

11.29

12.33

11.38

11.59 ± .60

$$\left(\frac{TL}{D}\right)_{150KVP} = \frac{11.59 \text{ mC}}{5.00 \text{ Gy}} = 2.32 \frac{\text{mC}}{\text{Gy}}$$

TLDs on March

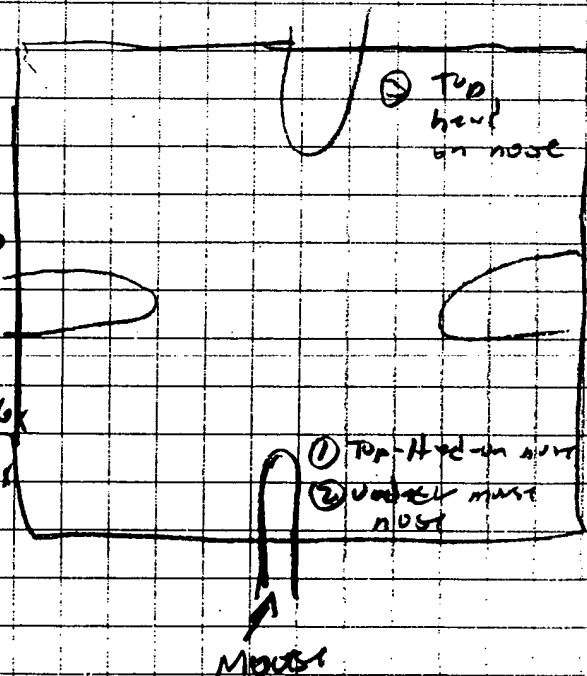
(1) 11.54 mC 497 cGy
 10.42 466 cGy 482 cGy

(2) 6.81 mC 294 cGy
 8.27 356

325 cGy

(3) 11.18 482 cGy
 9.60 414 cGy

448





The University of Chicago Departmental Purchase Order

Vendor Name Fisher Scientific
1600 W Glenlake Ave
Itasca, IL 60143
City Itasca State IL Zip Code 60143
Payment Terms Net 30 Delivery charge? ☐ Yes ☒ No
Telephone No. 2-3328 FAX No. 2-3328

Ship to
THE UNIVERSITY OF CHICAGO
Uof C Rad Onc
Department Rad Onc Dept. Code: 6-08
Greg Sibley
Room 5830 S. Ellis MC 0085
Chgo City IL State IL Zip Code 60637

Authorized Signature _____ Payroll No. _____
Print Name _____ Ext. _____
Account Code _____ Date _____
Order placed by phone? ☐ No ☒ Yes
Order placed by (name) Cindy Date 3/31/95
Order placed by (name) Greg Sibley Phone # 2-0294

QTY	UNIT	DESCRIPTION
4	ea	Hamilton Syringes 10 ml
2	Box	15ml polypropylene tubes 14-959-70C

DEPARTMENT COPY
NOT VALID IF TOTAL EXCEEDS \$500.00
BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

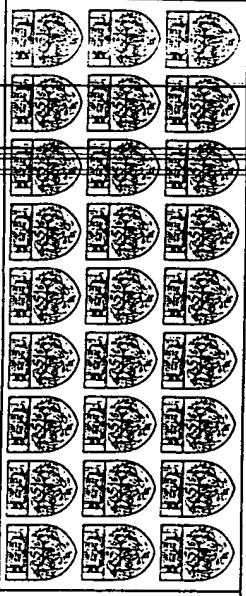
Purchase Order Number
Z890593
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.
Not to be used for purchase of travel, hazardous or radioactive materials,
controlled substances, vehicle rental or other restricted items.

IMPORTANT INFORMATION

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1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.



NET UNIT PRICE	ITEM TOTAL
23.04	92.16
83.40	166.80

ORDER TOTAL
NOT VALID IF TOTAL EXCEEDS \$500.00

INDUCTION-*in vivo*

RR
208
208

4/3/95

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0			weight	Day 3	weight
1720	AA105283	10^7 899-6	20.5	28	16.5	29.3		
1710	AA105283	10^7 899-6	0	0	0	23.6		
2075	AA105283	10^7 899-6	12	12	7	23.9		
1713	AA105283	10^7 899-6	0	0	0	21.6		
2052	AA111331	10^7 899-6	16.5	14.5	12	21.9		
2053	AA111331	10^7 899-6	21	15	11.5	24.1		
2054	AA111331	10^7 899-6	16	12	7.5	22.0		
2055	AA111331	10^7 899-6	23.5	21	10.5	22.75g	red	
2056	AA111332	10^7 899-6	20	18	11	25.9		
2057	AA111332	10^7 899-6	9.5	8	6	21.8		
2058	AA111332	10^7 899-6	19	20	8.5	25.9		
2076	AA111332	10^7 899-6	15	16.5	9	22.5		
2060	AA111333	10^7 899-6	19.5	18	13	25.8		
2061	AA111333	10^7 899-6	20	16	11.5	26.2		
2062	AA111333	10^7 899-6	13.5	8	8.5	17.2		
2063	AA111333	10^7 899-6	17.5	13	10.5	23.5		
		10^7 899-6						
1697	AA104549	10^7 3616	18.5	18.5	13	25.2		
1708	AA104549	10^7 3616	0	0	0	21.4		
1709	AA104549	10^7 3616	0	0	0	25.7		
1723	AA104549	10^7 3616	0	0	0	26.0		
2069	AA108968	10^7 3616	12	15	8	24.8		
2070	AA108968	10^7 3616	13	17	6	26.2		
2041	AA111328	10^7 3616	8	8	5.5	23.6		
2043	AA111328	10^7 3616	22	17	11.5	26.5		
2068	AA111328	10^7 3616	5	4.5	3	14.9		
2071	AA111328	10^7 3616	15	12.5	8	26.3		
2064	AA111334	10^7 3616	8.5	8	5	24.1		
2065	AA111334	10^7 3616	23	24	14	25.8		
2066	AA111334	10^7 3616	16	15.5	6.5	25.2		
2067	AA111334	10^7 3616	8	6	4.5	12.4		
90		10^7 3616						
1859	AA108972	RT Alone	9	9	4	25.5		
1861	AA108972	RT Alone	12	14.5	8	20.5		
1862	AA108972	RT Alone	6.5	8	5	24.9		
1892	AA108964	RT Alone	7	6.5	3.5	22.7		
		RT Alone						

4/3/95

1863	AA108971	10^7 3616+RT	8.5	7	3	23.4
1864	AA108971	10^7 3616+RT	6.5	4	4	24.4
1865	AA108971	10^7 3616+RT	2	2	1	24.1
1866	AA108971	10^7 3616+RT	3.5	3	1	25.0
1883	AA108966	10^7 3616+RT	0	0	0	22.2
1884	AA108966	10^7 3616+RT	4.5	4	2	22.7
1886	AA108966	10^7 3616+RT	10	10	4	14.6
2074	AA108966	10^7 3616+RT	0	0	0	22.3
2032	AA111326	10^7 3616+RT	7	5.5	5.5	19.5
2033	AA111326	10^7 3616+RT	11.5	8	6	21.2
2035	AA111326	10^7 3616+RT	10.5	8.5	7	18.3
2072	AA111326	10^7 3616+RT	4	5	2.5	22.1
2036	AA111327	10^7 3616+RT	11	8.5	5	21.0
2037	AA111327	10^7 3616+RT	6.5	5	3.5	24.5
2039	AA111327	10^7 3616+RT	12.5	11	6	18.2
2073	AA111327	10^7 3616+RT	7.5	7.5	7.5	18.9
		10^7 3616+RT				
1871	AA108969	10^7 899-6+RT	6	6	3	24.5
1872	AA108969	10^7 899-6+RT	6	4.5	3.5	23.4
1873	AA108969	10^7 899-6+RT	7	8.5	5	24.1
1874	AA108969	10^7 899-6+RT	5.5	3.5	2	25.8
1879	AA108967	10^7 899-6+RT	0	0	0	26.4
1880	AA108967	10^7 899-6+RT	0	0	0	22.1
1881	AA108967	10^7 899-6+RT	6	0	0	23.4
1882	AA108967	10^7 899-6+RT	0	0	0	24.7
2044	AA111329	10^7 899-6+RT	6.5	7.5	4	23.2
2045	AA111329	10^7 899-6+RT	3	3.5	1.5	25.2
2046	AA111329	10^7 899-6+RT	6.5	6	4	24.0
2047	AA111329	10^7 899-6+RT	0	0	0	22.4
2048	AA111330	10^7 899-6+RT	4.5	3.5	2.5	20.0
2049	AA111330	10^7 899-6+RT	12.5	11	7	23.4
2050	AA111330	10^7 899-6+RT	6	5	4	23.5
2051	AA111330	10^7 899-6+RT	9	6.5	4	23.2
		10^7 899-6+RT				

1863	AA108971	10^7 3616+RT	8.5	7	4
1864	AA108971	10^7 3616+RT	8.5	6	5
1865	AA108971	10^7 3616+RT	3	3	1
1866	AA108971	10^7 3616+RT	2.5	2	+ 0 0 0
1883	AA108966	10^7 3616+RT	5	4	1
1884	AA108966	10^7 3616+RT	3.5	3	2.5
1886	AA108966	10^7 3616+RT	0	0	0
2072	AA108966	10^7 3616+RT	9.5	10.5	4.5
2032	AA111326	10^7 3616+RT	6.5	5	4.5
2033	AA111326	10^7 3616+RT	11	8	6
2035	AA111326	10^7 3616+RT	11	8.5	6.5
2072	AA111326	10^7 3616+RT	6	4	1
2036	AA111327	10^7 3616+RT	10	9.5	4.5
2037	AA111327	10^7 3616+RT	4.5	6	2.5
2039	AA111327	10^7 3616+RT	10	12	7.5
2073	AA111327	10^7 3616+RT	7	6.5	3
		10^7 3616+RT			
1871	AA108969	10^7 899-6+RT	6.5	6	3
1872	AA108969	10^7 899-6+RT	6.4	4.6	3.5
1873	AA108969	10^7 899-6+RT	7	7.5	4.5
1874	AA108969	10^7 899-6+RT	3	3.5	1.5
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	4	5.5	1.5
1881	AA108967	10^7 899-6+RT	4	3	1
1882	AA108967	10^7 899-6+RT	0	0	0
2044	AA111329	10^7 899-6+RT	7.5	6	2.5 6 6.5 4
2045	AA111329	10^7 899-6+RT	3.5	3	1
2046	AA111329	10^7 899-6+RT	2.5	6	3.5
2047	AA111329	10^7 899-6+RT	3	3	1
2048	AA111330	10^7 899-6+RT	4	4	3
2049	AA111330	10^7 899-6+RT	13	11	7
2050	AA111330	10^7 899-6+RT	3	5	2.5 edema
2051	AA111330	10^7 899-6+RT	6.5	9	4
		10^7 899-6+RT			
2082	AA111338	S brain 2P	1000 x 5	died	
2083	AA111339	S "	500 x 8		
2084	AA111339	S "	500 x 6		
2085	AA111339	S stereoback			
2086	AA111339	S "			
2087	AA111340	S "			
2088	AA111340	S "			
2089	AA111340	S			
2090	AA111340	S			

2082	AA111338	S	brain 2P	1000 x 5	died
2083	AA111339	S	"	500 x 8	
2084	AA111339	S	"	500 x 6	
2085	AA111339	S	7 stereoblocks		
2086	AA111339	S	"		
2087	AA111340	S	"		
2088	AA111340	S	"		
2089	AA111340	S			
2090	AA111340	S			

4/6/95

Greg's Mouse Log

Mouse #	Cage #	Group			
710	AA105283	10^7 899-6	0	0	0
2075	AA105283	10^7 899-6	16	14	8.5
1713	AA105283	10^7 899-6	0	0	0
2052	AA111331	10^7 899-6	23	17.5	13
2053	AA111331	10^7 899-6	18.5	12	13
2054	AA111331	10^7 899-6	28	14.5	13
2056	AA111332	10^7 899-6	21.5	15.5	12
2057	AA111332	10^7 899-6	9.5	11	6.5
2058	AA111332	10^7 899-6	19.5	21.5	10.5
2076	AA111332	10^7 899-6	17	18.5	9.5
2061	AA111333	10^7 899-6	23	18.5	12.5
2062	AA111333	10^7 899-6	15.5	11	10
2063	AA111333	10^7 899-6	20.5	16	11.5
		10^7 899-6			

sacked 4/6

16.5 12 8.5

1697	AA104549	10^7 3616	18.5	19	12
1708	AA104549	10^7 3616	0	0	0
1709	AA104549	10^7 3616	0	0	0
1723	AA104549	10^7 3616	0	0	0
2069	AA108968	10^7 3616	13.5	17	10
2070	AA108968	10^7 3616	13	16	17.5
2041	AA111328	10^7 3616	10.5	9.5	6.5
2068	AA111328	10^7 3616	4.5	4	2.5
2071	AA111328	10^7 3616	18	16.5	10
2064	AA111334	10^7 3616	9	10	7
2066	AA111334	10^7 3616	20	17	9
		10^7 3616			

7.5 remeasure

1859	AA108972	RT Alone	10	10	4
1861	AA108972	RT Alone	12	13	10.5
1862	AA108972	RT Alone	6.5	7	4
1892	AA108964	RT Alone	7.5	7.5	3.5
		RT Alone			

18.5
16.5
14.5
12.5

[illegible]

April 12, 1995

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

We placed our mice on Bactrim in Cummings Rm #1053 (on 4/12/95) due to the appearance of declining health and a number of deaths after tumor implantation. I apologize for not making you aware of this. Please continue Bactrim therapy for 1 week (until 4/18/95).

The mice placed on antibiotics include all mice with HALLAHAN/SIBLEY on the card (20 cages).

Cage #'s:

Sorry for the confusion.

Sincerely,

Gregory S. Sibley, M.D.

4/13

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	21.5	19.5	9					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	11	14	4.5					
2057	AA111332	10^7 899-6	13	10	7					
1709	AA104549	10^7 3616	0	0	0					
1723	AA104549	10^7 3616	0	0	0					
2069	AA108968	10^7 3616	23	20	13.5					
2070	AA108968	10^7 3616	23	19	11					
2064	AA111334	10^7 3616	15	14	7.5					
2041	"	10^7 3616	12	11	9					
1859	AA108972	RT Alone	11	10	6					
1861	AA108972	RT Alone	13.5	12.5	8					
1862	AA108972	RT Alone	6	6	3.5					
1892	AA108964	RT Alone	6	6	3.5					
1863	AA108971	10^7 3616+RT	8	6	4					
1864	AA108971	10^7 3616+RT	12	10	9					
1865	AA108971	10^7 3616+RT	0	0	0					
1866	AA108971	10^7 3616+RT	0	0	0					
1883	AA108966	10^7 3616+RT	0	0	0					
1884	AA108966	10^7 3616+RT	8	3.5	2					
1886	AA108966	10^7 3616+RT	9	0	0					
2074	AA108966	10^7 3616+RT	9	10	5					
2032	AA111326	10^7 3616+RT	6	5	5					
2033	AA111326	10^7 3616+RT	9	7	3.5					
2035	AA111326	10^7 3616+RT	8.9	7	6					
2072	AA111326	10^7 3616+RT	0	0	0					
2036	AA111327	10^7 3616+RT	10	9	4					
2037	AA111327	10^7 3616+RT	5	3.5	2.5					
2039	AA111327	10^7 3616+RT	7	8	3.5					
2073	AA111327	10^7 3616+RT	9.5	9	5					
1871	AA108969	10^7 899-6+RT	6.5	6.5	4					
1872	AA108969	10^7 899-6+RT	4	5	3.5					
1873	AA108969	10^7 899-6+RT	7	7	4.5					
1874	AA108969	10^7 899-6+RT	0	0	0					
1879	AA108967	10^7 899-6+RT	0		0					
1880	AA108967	10^7 899-6+RT	0		0					
1881	AA108967	10^7 899-6+RT	0		0					
1882	AA108967	10^7 899-6+RT	0		0					
2044	AA111329	10^7 899-6+RT	4.5	4.5	3.5					
2045	AA111329	10^7 899-6+RT	0	0	0					
2046	AA111329	10^7 899-6+RT	6.5	6	3					
2047	AA111329	10^7 899-6+RT	0	0	0					
2048	AA111330	10^7 899-6+RT	3	3.5	2					
2049	AA111330	10^7 899-6+RT	14	12	10					
2050	AA111330	10^7 899-6+RT	3	2.5	1					
2051	AA111330	10^7 899-6+RT	9	6.5	4.5					

4/17/95

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10 ⁺ 7 899-6	0	0	0	23.0				
2075	AA105283	10 ⁺ 7 899-6	21	18	6.5	21.9				
1713	AA105283	10 ⁺ 7 899-6	0	0	0	21.6				
2054	AA111331	10 ⁺ 7 899-6	11	15	4	19.2				
2057	AA111332	10 ⁺ 7 899-6	14.5	12	8.5	21.1				
1709	AA104549	10 ⁺ 7 3616	0	0	0	23.9				
1723	AA104549	10 ⁺ 7 3616	0	0	0	24.9				
2069	AA108968	10 ⁺ 7 3616								
2070	AA108968	10 ⁺ 7 3616								
2064	AA111334	10 ⁺ 7 3616	15.5	14	8	23.4				
2041		3616	13.5	12	9	21.9				
1859	AA108972	RT Alone	11.5	10	5	26.1				
1861	AA108972	RT Alone	6.5	6	4.5	24.9				
1862	AA108972	RT Alone	14	14	7.5	22.5				
1892	AA108964	RT Alone	6.5	6.5	3.5	22.8				
1863	AA108971	10 ⁺ 7 3616+RT	8	5	3	24.5				
1864	AA108971	10 ⁺ 7 3616+RT	12	11	8.5	25.6				
1865	AA108971	10 ⁺ 7 3616+RT	0	0	0	26.4				
1866	AA108971	10 ⁺ 7 3616+RT	0	0	0	25.8				
1883	AA108966	10 ⁺ 7 3616+RT	0	0	0	22.0				
1884	AA108966	10 ⁺ 7 3616+RT	3	3	2	22.3				
1886	AA108966	10 ⁺ 7 3616+RT	10	9	4.5	16.4				
2074	AA108966	10 ⁺ 7 3616+RT	0	0	0	21.5				
2032	AA111326	10 ⁺ 7 3616+RT	6	5	5	19.1				
2033	AA111326	10 ⁺ 7 3616+RT	6	5.5	3.5	22.4				
2035	AA111326	10 ⁺ 7 3616+RT	8	6	4	19.3				
2072	AA111326	10 ⁺ 7 3616+RT	0	0	0	22.7				
2036	AA111327	10 ⁺ 7 3616+RT	10	9	4	22.5				
2037	AA111327	10 ⁺ 7 3616+RT	4	5	2.5	24.0				
2039	AA111327	10 ⁺ 7 3616+RT	8	8	4	18.4				
2073	AA111327	10 ⁺ 7 3616+RT	8	9	5	19.4				
1871	AA108969	10 ⁺ 7 899-6+RT	5	5	2	25.4				
1872	AA108969	10 ⁺ 7 899-6+RT	6.5	5.5	3	22.6				
1873	AA108969	10 ⁺ 7 899-6+RT	7	7.5	5	23.3				
1874	AA108969	10 ⁺ 7 899-6+RT	0	0	0	26.1				
1879	AA108967	10 ⁺ 7 899-6+RT	0	0	0	28.7				
1880	AA108967	10 ⁺ 7 899-6+RT	0	0	0	21.9				
1881	AA108967	10 ⁺ 7 899-6+RT	0	0	0	25.6				
1882	AA108967	10 ⁺ 7 899-6+RT	0	0	0	23.6				
2044	AA111329	10 ⁺ 7 899-6+RT	4	4	3.5	22.8				
2045	AA111329	10 ⁺ 7 899-6+RT	0	0	0	25.6				
2046	AA111329	10 ⁺ 7 899-6+RT	0.5	6	3	23.4				
2047	AA111329	10 ⁺ 7 899-6+RT	0	0	0	21.7				
2048	AA111330	10 ⁺ 7 899-6+RT	4	3	2	18.3				
2049	AA111330	10 ⁺ 7 899-6+RT	14	13	6	22.6				
2050	AA111330	10 ⁺ 7 899-6+RT	3	3	2	22.7				
2051	AA111330	10 ⁺ 7 899-6+RT	8	10	3.5	21.7				

4/20

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	φ							
2075	AA105283	10^7 899-6	20	15.5	6					
1713	AA105283	10^7 899-6	φ							
2054	AA111331	10^7 899-6	14	9.5	4					
2057	AA111332	10^7 899-6	15	11.5	8					
1709	AA104549	10^7 3616	φ							
1723	AA104549	10^7 3616	φ							
2069	AA108968	10^7 3616								
2070	AA108968	10^7 3616								
2064	AA111334	10^7 3616	15.5	13.5	10.5					
2041	AA111334	10^7 3616	14	12	9					
1859	AA108972	RT Alone	11	9.5	7					
1861	AA108972	RT Alone face	11	9.5	7	14.5	13	12.5		
1862	AA108972	RT Alone	5.5	5	3.5					
1892	AA108964	RT Alone neotenic tail	6.5	6	3.5					
1863	AA108971	10^7 3616+RT	6	5	4					
1864	AA108971	10^7 3616+RT	11	10	9.5					
1865	AA108971	10^7 3616+RT	φ							
1866	AA108971	10^7 3616+RT	φ							
1883	AA108966	10^7 3616+RT	φ							
1884	AA108966	10^7 3616+RT	2	2	2					
1886	AA108966	10^7 3616+RT	φ							
2074	AA108966	10^7 3616+RT	8.5	10	5					
2032	AA111326	10^7 3616+RT	4.5	5	4.5					
2033	AA111326	10^7 3616+RT	5.5	5	3					
2035	AA111326	10^7 3616+RT	8	7	5					
2072	AA111326	10^7 3616+RT	φ							
2036	AA111327	10^7 3616+RT	8.5	8	5					
2037	AA111327	10^7 3616+RT	4.5	4	2.5					
2039	AA111327	10^7 3616+RT	8	7	6					
2073	AA111327	10^7 3616+RT	6.5	7	3					
1871	AA108969	10^7 899-6+RT	φ							
1872	AA108969	10^7 899-6+RT	2.5	2.5	2					
1873	AA108969	10^7 899-6+RT	7	7.5	6					
1874	AA108969	10^7 899-6+RT	φ							
1879	AA108967	10^7 899-6+RT								
1880	AA108967	10^7 899-6+RT	φ							
1881	AA108967	10^7 899-6+RT	φ							
1882	AA108967	10^7 899-6+RT	φ							
2044	AA111329	10^7 899-6+RT	4.5	4	2.5					
2045	AA111329	10^7 899-6+RT	φ							
2046	AA111329	10^7 899-6+RT	6.5	6	3					
2047	AA111329	10^7 899-6+RT	φ							
2048	AA111330	10^7 899-6+RT	3	3	2					
2049	AA111330	10^7 899-6+RT	10	9	6	13	12	10		
2050	AA111330	10^7 899-6+RT	10	9	6					
2051	AA111330	10^7 899-6+RT	10	9	6					

4/24/95

Mouse #	Cage #	Group	Day 0	weight	Day 3	weight
1710	AA105283	10^7 899-6	0 0 0	23.3		
2075	AA105283	10^7 899-6	21 17 8	22.4		
1713	AA105283	10^7 899-6	0 0 0	21.9		
2054	AA111331	10^7 899-6	13.5 6.5 5	20.5		
2057	AA111332	10^7 899-6	18.5 16.5 14.5	23.3		
1709	AA104549	10^7 3616	0 0 0	24.0		
1723	AA104549	10^7 3616	0 0 0	23.5		
2041	AA111334	10^7 3616	18 16 14	24.8		
2064	AA111334	10^7 3616	18.5 16.5 12	23.5		
1859	AA108972	RT Alone	12 9.5 8.5	26.1		
1861	AA108972	RT Alone <i>gac</i>				
1862	AA108972	RT Alone	5.5 4.5 3.5	25.1		
1892	AA108964	RT Alone	5.5 5.5 3.5	24.1		
1863	AA108971	10^7 3616+RT	5 4.5 3.5	25.0		
1864	AA108971	10^7 3616+RT	11 14 11	25.1		
1865	AA108971	10^7 3616+RT	0 0 0	25.9		
1866	AA108971	10^7 3616+RT	0 0 0	25.3		
1883	AA108966	10^7 3616+RT	0 0 0	22.2		
1884	AA108966	10^7 3616+RT	2 2 1	24.4		
1886	AA108966	10^7 3616+RT	0 0 0	21.3		
2074	AA108966	10^7 3616+RT	8 9 6	16.3		
2032	AA111326	10^7 3616+RT	4.5 5.5 3.5	22.0		
2033	AA111326	10^7 3616+RT	5.5 4.5 3.5	24.8		
2035	AA111326	10^7 3616+RT	8 6.5 5	21.7		
2072	AA111326	10^7 3616+RT	0 0 0	24.2		
2036	AA111327	10^7 3616+RT	8 7 4	23.9		
2037	AA111327	10^7 3616+RT	4 4.5 1.5	24.6		
2039	AA111327	10^7 3616+RT	9 9 8	20.3		
2073	AA111327	10^7 3616+RT	7 7 4	20.2		
1871	AA108969	10^7 899-6+RT	5 5 3	23.3		
1872	AA108969	10^7 899-6+RT	<i>gac</i>			
1873	AA108969	10^7 899-6+RT	6.5 8 5.5	23.8		
1874	AA108969	10^7 899-6+RT	0 0 0	26.1		
1880	AA108967	10^7 899-6+RT	0 0 0	22.4		
1881	AA108967	10^7 899-6+RT	0 0 0	25.4		
1882	AA108967	10^7 899-6+RT	0 0 0	29.4		
2044	AA111329	10^7 899-6+RT	3.5 3.5 3	23.3		
2045	AA111329	10^7 899-6+RT	0 0 0	25.9		
2046	AA111329	10^7 899-6+RT	5.5 6 2.5	24.8		
2047	AA111329	10^7 899-6+RT	0 0 0	22.1		
2048	AA111330	10^7 899-6+RT	2.5 2.0 1	17.4		
2049	AA111330	10^7 899-6+RT	13.5 11 9	24.7		
2050	AA111330	10^7 899-6+RT	4 3 2	23.2		
2051	AA111330	10^7 899-6+RT	8.5 8.0 6.5	22.9		

gac

7/27/95

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	16	12	3					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	11	5	3.5					
2057	AA111332	10^7 899-6	20	17.5	15					
1709	AA104549	10^7 3616	0	0	0					
1723	AA104549	10^7 3616	0	0	0					
2041	AA111334	10^7 3616	19	17	15					
2064	AA111334	10^7 3616	17.5	17	13.5					
1859	AA108972	RT Alone	12	11	8					
1861	AA108972	RT Alone	5	4	3.5					
1862	AA108972	RT Alone	5	4	3.5					
1892	AA108964	RT Alone	6	5	4					
1863	AA108971	10^7 3616+RT	5	5	3.5					
1864	AA108971	10^7 3616+RT	13	4.5	5.5					
1865	AA108971	10^7 3616+RT	0	0	0					
1866	AA108971	10^7 3616+RT	0	0	0					
1883	AA108966	10^7 3616+RT	0	0	0					
1884	AA108966	10^7 3616+RT	2	2.5	1					
1886	AA108966	10^7 3616+RT	0	0	0					
2074	AA108966	10^7 3616+RT	9.5	8.5	5					
2032	AA111326	10^7 3616+RT	4.5	4	3.5					
2033	AA111326	10^7 3616+RT	5.5	5	3					
2035	AA111326	10^7 3616+RT	8	6.5	4.5					
2072	AA111326	10^7 3616+RT	0	0	0					
2036	AA111327	10^7 3616+RT	8	7	4					
2037	AA111327	10^7 3616+RT	4	4	2					
2039	AA111327	10^7 3616+RT	9.5	8	6					
2073	AA111327	10^7 3616+RT	7	7	3					
1871	AA108969	10^7 899-6+RT	5.5	5	3					
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	2.5	6	5					
1874	AA108969	10^7 899-6+RT	0	0	0					
1880	AA108967	10^7 899-6+RT	0	0	0					
1881	AA108967	10^7 899-6+RT	0	0	0					
1882	AA108967	10^7 899-6+RT	0	0	0					
2044	AA111329	10^7 899-6+RT	4	3.5	3					
2045	AA111329	10^7 899-6+RT	0	0	0					
2046	AA111329	10^7 899-6+RT	5	5	2.5					
2047	AA111329	10^7 899-6+RT								
2048	AA111330	10^7 899-6+RT	2	3	1.5					
2049	AA111330	10^7 899-6+RT	16	13	10					
2050	AA111330	10^7 899-6+RT	2	3	2					
2051	AA111330	10^7 899-6+RT	9	8	5.5					

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17
126

306
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14

5/1/95

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0	24.8				
2075	AA105283	10^7 899-6	0	0	0	21.8				
1713	AA105283	10^7 899-6	3	2	1	21.8				
2054	AA111331	10^7 899-6	0	0	0	21.8				
2057	AA111332	10^7 899-6								
1709	AA104549	10^7 3616								
1723	AA104549	10^7 3616	0	0	0	23.7				
2041	AA111334	10^7 3616								
2064	AA111334	10^7 3616								
1859	AA108972	RT Alone	13	11.5	8	23.9				
1861	AA108972	RT Alone								
1862	AA108972	RT Alone	4.5	5	4	25.8				
1892	AA108964	RT Alone	5	5.5	4	24.3				
1863	AA108971	10^7 3616+RT	4.5	4	3	24.6				
1864	AA108971	10^7 3616+RT	13	10.5	7.5	26.3				
1865	AA108971	10^7 3616+RT	2.5	2.5	1	26.5				
1866	AA108971	10^7 3616+RT	3	3.5	1	25.8				
1883	AA108966	10^7 3616+RT	0	0	0	22.6				
1884	AA108966	10^7 3616+RT	1.5	1.5	1.5	24.3				
1886	AA108966	10^7 3616+RT	0	0	0	21.4				
2074	AA108966	10^7 3616+RT	9.5	8	6	17.3				
2032	AA111326	10^7 3616+RT	7	5	4	21.4				
2033	AA111326	10^7 3616+RT	4.5	4	3	24.3				
2035	AA111326	10^7 3616+RT	8	7	5.5	21.4				
2072	AA111326	10^7 3616+RT	0	0	0	23.5				
2036	AA111327	10^7 3616+RT	7	7	3	22.9				
2037	AA111327	10^7 3616+RT	3.5	3	2	24.7				
2039	AA111327	10^7 3616+RT	9	8	6.5	18.3				
2073	AA111327	10^7 3616+RT	6	5.5	2	19.5				
1871	AA108969	10^7 899-6+RT	4	4	3	23.7				
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	6.5	6.5	3	23.6				
1874	AA108969	10^7 899-6+RT	0	0	0					
1880	AA108967	10^7 899-6+RT	0	0	0	21.8				
1881	AA108967	10^7 899-6+RT	0	0	0	26.6				
1882	AA108967	10^7 899-6+RT	0	0	0	23.5				
2044	AA111329	10^7 899-6+RT	4	3.5	2.5	22.9				
2045	AA111329	10^7 899-6+RT	0	0	0	25.9				
2046	AA111329	10^7 899-6+RT	4.5	4	3	23.8				
2047	AA111329	10^7 899-6+RT								
2048	AA111330	10^7 899-6+RT	3.5	3	1.5	16.8				
2049	AA111330	10^7 899-6+RT	15.5	13.5	11	26.4				
2050	AA111330	10^7 899-6+RT	2.5	2	1	23.2				
2051	AA111330	10^7 899-6+RT	9	7.5	5	22.6				

4/12/95

Apoptosis Assay

Purpose - To determine whether apoptosis plays a role in V-87 cell killing with TNF, RT, R3616 virus or R899-6 virus or combinations of these

Methods - 8 T150 flasks of V87 cells were grown to ~80% confluence.

	7 ^{pm}	1 ^{pm}	5 ^{pm}	1 ^{pm}			
	-6°	-4°	-2°	0°	2°	4°	→ 24°
Control (no Rx)							Harvest
TNF (100 ¹³ μM)				TNF			Harvest
RT (20 Gr) + TNF				TNF		RT	Harvest
RT (20 Gr)						RT	Harvest
3.8 ml R3616 (10 ⁷ PFU) + RT	infect					RT	Harvest
R3616 (10 ⁷ PFU)	infect						Harvest
2.6 ml R899-6 (10 ⁷ PFU) + RT	infect					RT	Harvest
R899-6 (10 ⁷ PFU)	infect						Harvest

1. Virally infected cells flasks were ~~to~~ treated with 10^7 PFU of virus (mol approx 0.5) in 6 ml 199V media. Other flasks incubated 6 ml of 199V ~~5~~ virus as control.

2. At 2 hrs, an additional ¹⁴ ~~24~~ ml of 20% FCS media was added to bring total volume to ²⁰ ~~30~~ cc. (Actually I added 10% FCS by mistake x 3 hrs, switched to 0% FCS @ 12^h, this required decanting media from infected flasks)

3. Add TNF to appropriate flasks = .01 mg/ml stock = .01 ¹⁰ μ g/ μ l add 10 μ l (100 ng) to 20 ml

APOPTOSIS ASSAY

4/24/95

Sample	Apoptosis (%)
Baseline	2.3
Positive Control	80.4
No Treatment (-TdT)	0.3
No Treatment	1.9
RT 20 Gy	19.3
TNF	2.6
TNF + RT	19
R3616 (MOI=0.5)	29.3
R3616 + RT	23.2
R899-6 (MOI=0.5)	26.8
R899-6 + RT	11.4

U87

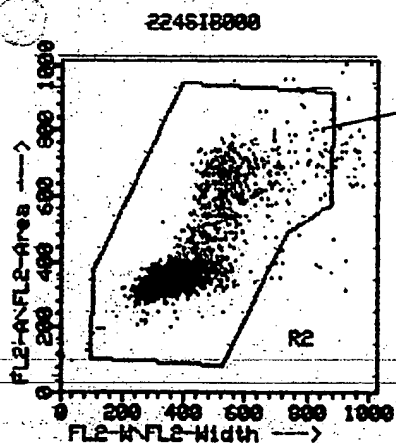
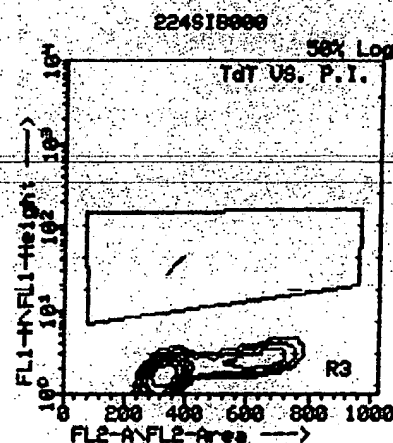
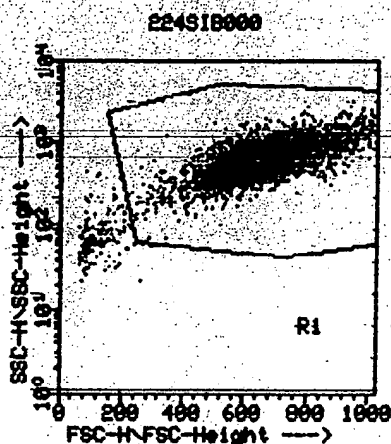
Neg. Control
No Td TBECTON
DICKINSON

LYSYS II Ver 1.1 2/5/92

DATE: 24-APR-95

TIME: 14:54:28

SELECTED PREFERENCES: Arithmetic/Linear



224SIB000

Region Stats

File: 224SIB000 Sample: GREG SIBLE

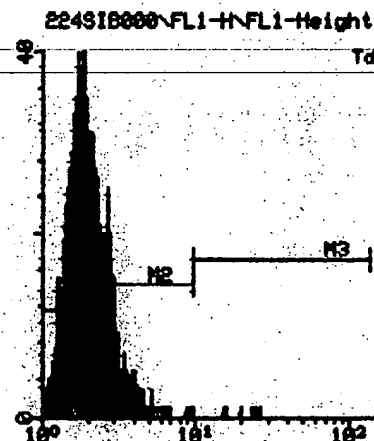
Date: 4/24/95 Gate G2= R1AND2

Selected Preferences Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2368

Rgn	Events	% Gated	% Total
1 R1	2368	100.00	78.93
2 R2	2368	100.00	78.93
3 R3	8	0.34	0.27



224SIB000\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 224SIB000

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1AND2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2368	100.00	43	1.91
1	1.00, 2.01	2100	89.02	43	1.04
2	2.01, 9.02	273	11.53	20	3.16
3	9.02, 136	7	0.30	1	19.01

CONTROL

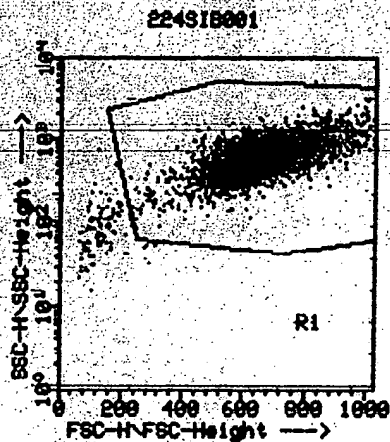
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

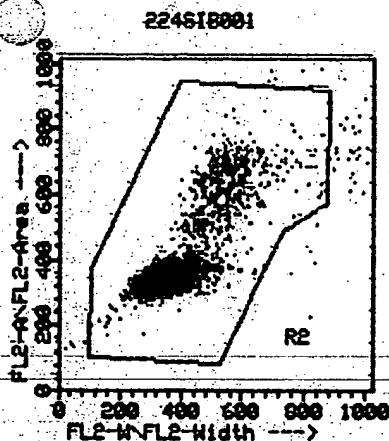
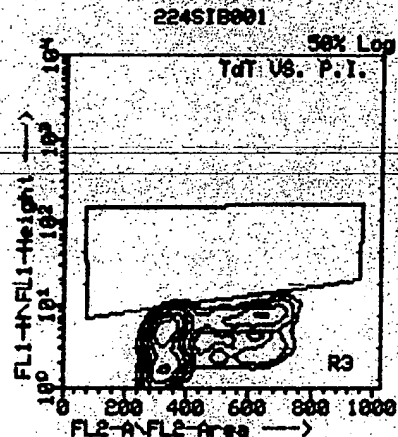
DATE: 24-APR-95

TIME: 14:56:24

SELECTED PREFERENCES: Arithmetic/Linear



*serum
depleted*



224SIB001

Region Stats

File: 224SIB001 Sample: GRED SIBL

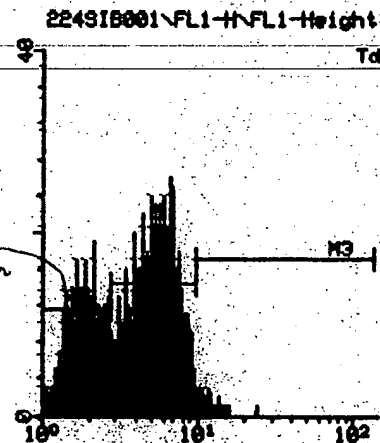
Date: 4/24/95 Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Line

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2514

Rgn	Events	% Gated	% Total
1 R1	2514	100.00	83.80
2 R2	2514	100.00	83.80
3 R3	13	0.52	0.43



*Monoclonal
combination*

224SIB001 \ FL1-H \ FL1-Height

Arithmetic Histogram Statistics for 224SIB001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H \ FL1-Height Gate G2= R1ANDR2

N	Left,Right	Events	%	Peak	Median
0	1.00, 9918	2514	100.00	26	4.33
1	1.00, 2.01	819	32.58	19	1.91
2	2.01, 9.02	1657	65.91	26	5.33
3	9.02, 136	48	1.91	3	11.09

RT

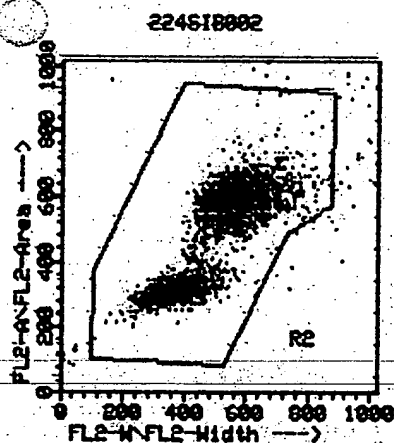
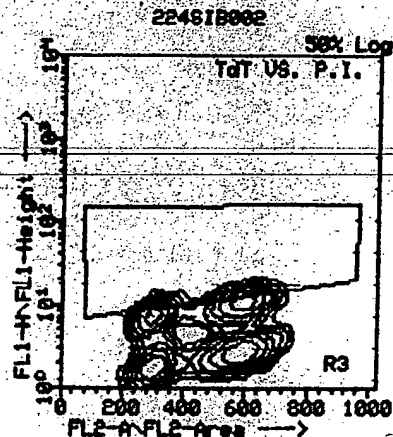
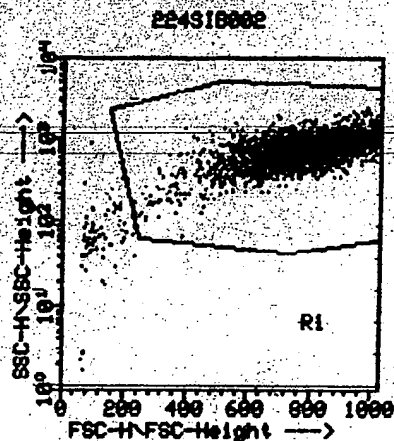
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95

TIME: 14:58:36

SELECTED PREFERENCES: Arithmetic/Linear



224SIB002

Region Stats

File: 224SIB002 Sample: GREG SIBLE

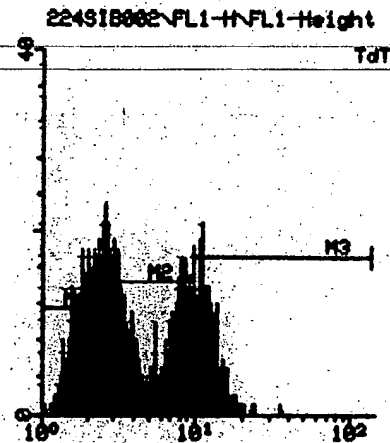
Date: 4/24/95 Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Log

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2343

Rgn	Events	% Gated	% Total
1 R1	2343	100.00	78.10
2 R2	2343	100.00	78.10
3 R3	289	8.92	6.97



224SIB002-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 224SIB002

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2343	100.00	23	3.34
1	1.00, 2.81	937	39.99	23	2.15
2	2.81, 9.82	974	41.57	19	5.03
3	9.82, 136	451	19.25	21	11.06

TNF-RT

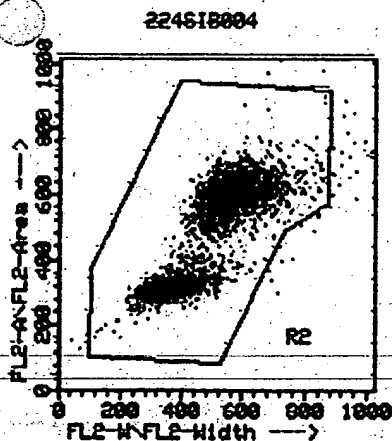
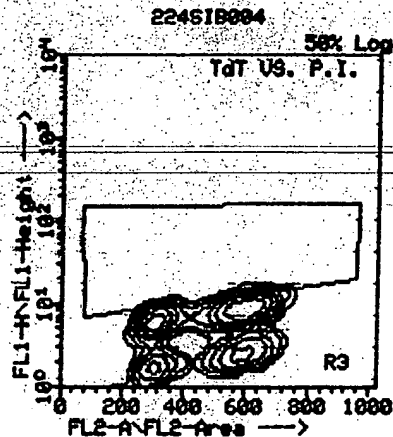
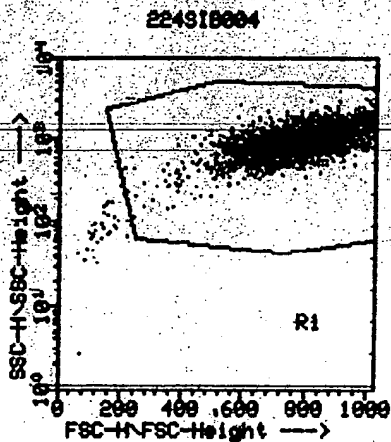
BECKTON
DICKINSON

LYSYS II Ver 1.1 2/5/92

DATE: 24-APR-95

TIME: 15:01:05

SELECTED PREFERENCES: Arithmetic/Linear



224SIB004

Region Stats

File: 224SIB004 Sample: GREG SIBLE

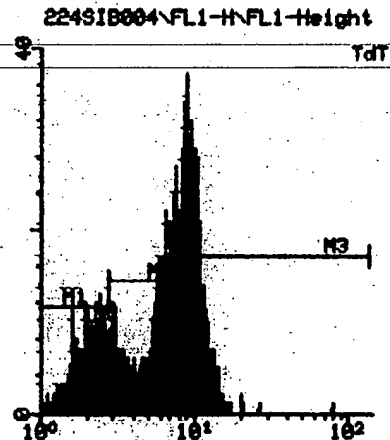
Date: 4/24/95 Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2530

Rgn	Events	% Gated	% Total
1 R1	2530	100.00	84.33
2 R2	2530	100.00	84.33
3 R3	61	2.41	2.03



224SIB004\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 224SIB004

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2530	100.00	37	7.17
1	1.00, 2.01	511	20.20	13	2.87
2	2.01, 9.02	1571	62.09	37	7.30
3	9.02, 136	401	19.01	29	10.75

3616

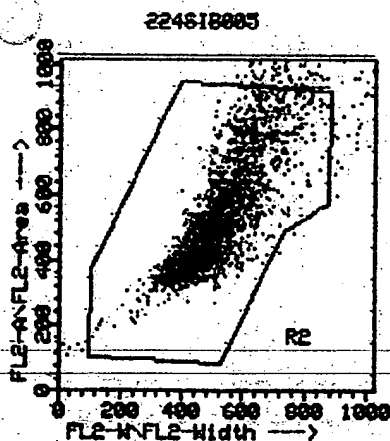
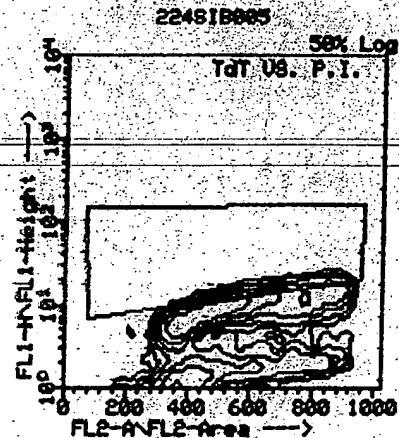
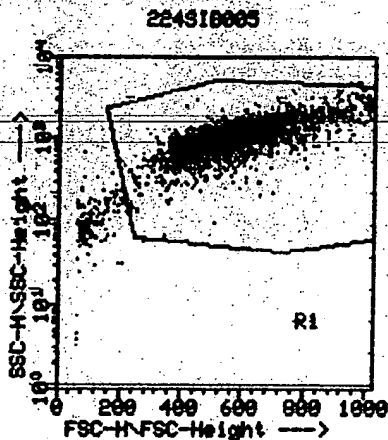
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95

TIME: 15:01:58

SELECTED PREFERENCES: Arithmetic/Linear



224SIB005

Region Stats

File: 224SIB005 Sample: GREG SIBLE

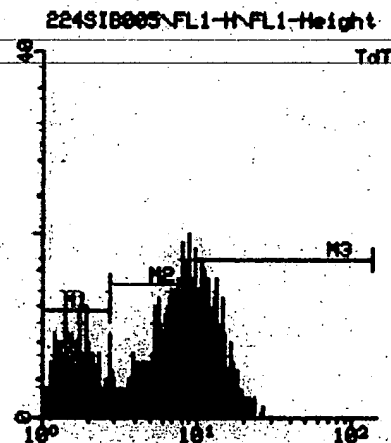
Date: 4/24/95 Gate G2= RIANDR2

Selected Preference: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2002

Rgn	Events	% Gated	% Total
1 R1	2002	100.00	66.73
2 R2	2002	100.00	66.73
3 R3	189	9.44	6.30



224SIB005\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 224SIB005

Selected Preference: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2002	100.00	20	7.17
1	1.00, 2.01	524	26.17	20	1.61
2	2.01, 9.02	989	49.40	20	6.90
3	9.02, 136	507	25.32	17	12.30

899-6

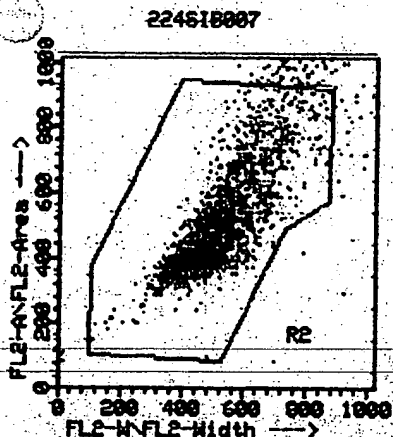
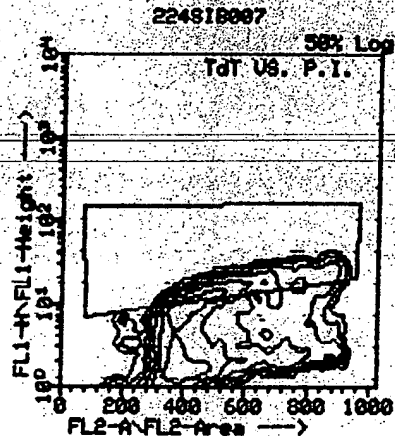
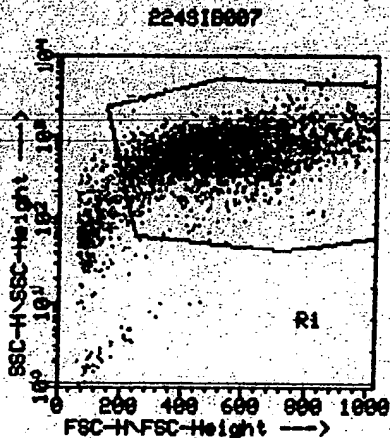
BECTION
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95

TIME: 15:03:40

SELECTED PREFERENCES: Arithmetic/Linear



224SIB007

Region Stats

File: 224SIB007 Sample: GREG SIBLE

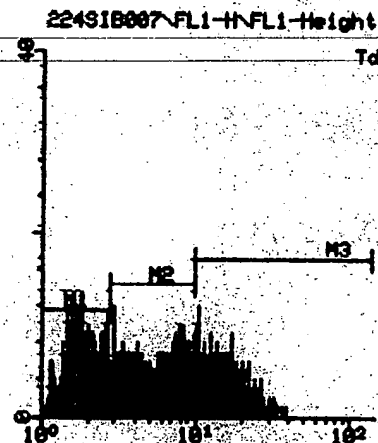
Date: 4/24/95 Gate G2= RIANDR2

Selected Preference: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(L00)

Total= 3000 Gated= 1710

Rgn	Events	% Gated	% Total
1 R1	1710	100.00	57.00
2 R2	1710	100.00	57.00
3 R3	366	21.40	12.20



224SIB007 FL1-H FL1-Height

Arithmetic Histogram Statistics for 224SIB007

Selected Preference: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1710	100.00	13	4.49
1	1.00, 2.81	599	35.03	13	1.04
2	2.81, 9.82	658	38.48	12	5.33
3	9.82, 136	459	26.64	12	14.20

899-6 + RT

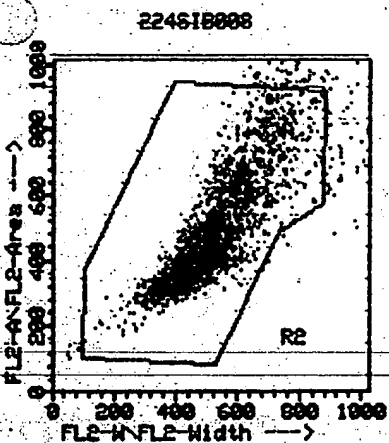
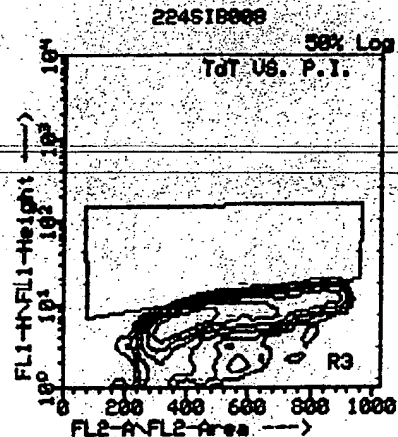
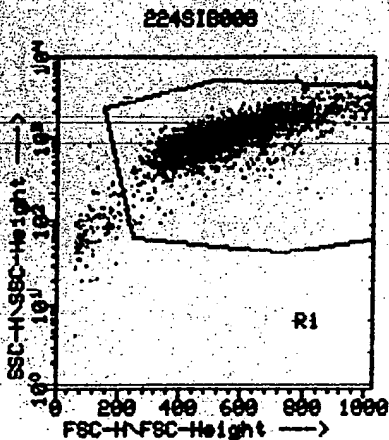
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95

TIME: 15:04:34

SELECTED PREFERENCES: Arithmetic/Linear



224SIB008

----- Region Stats -----

File: 224SIB008 Sample: GREG SIBLE

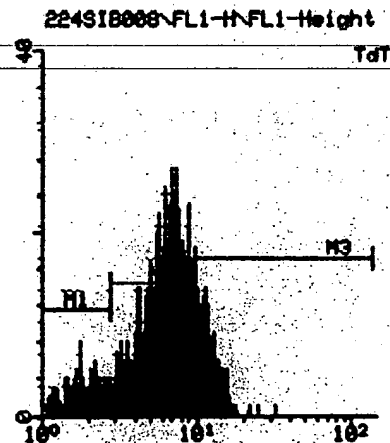
Date: 4/24/95 Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2068

Rgn	Events	% Gated	% Total
1 R1	2068	100.00	68.93
2 R2	2068	100.00	68.93
3 R3	30	1.45	1.00



224SIB008 FL1-H-FL1-Height

----- Arithmetic Histogram Statistics for 224SIB008 -----

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left, Right	Events	%	Peak	Median
0	1.00, 9910	2068	100.00	27	6.73
1	1.00, 2.01	210	10.54	8	1.04
2	2.01, 9.02	1515	73.26	27	6.49
3	9.02, 136	349	16.00	14	11.44

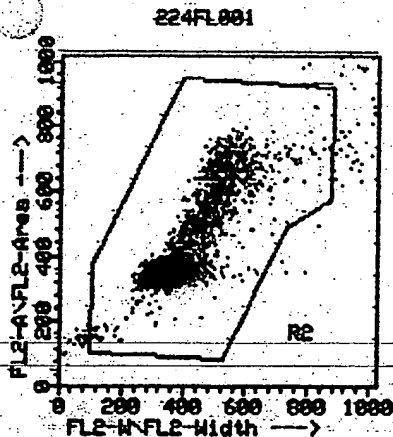
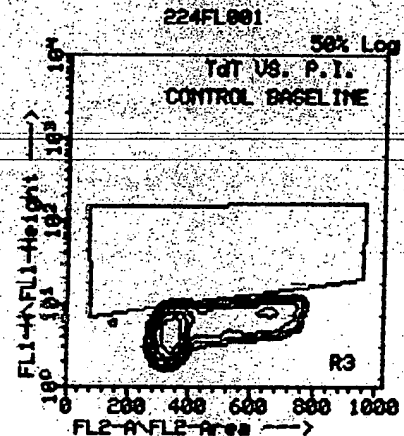
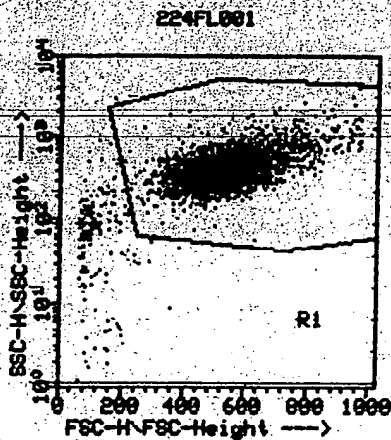
**BECTON
DICKINSON**

LYSYS II Ver 1.1 2/6/92 *Baseline for Positive Control*

DATE: 24-APR-95

TIME: 15:11:28

SELECTED PREFERENCES: Arithmetic/Linear



224FL001

Region Stats

File: 224FL001 Samples: FL5.12 CONTI

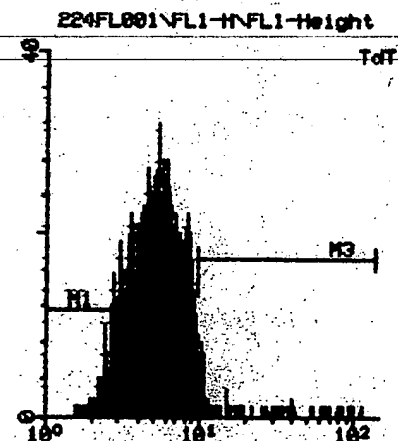
Date: 4/24/95 Gate G2= RIANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2487

Rgn	Events	% Gated	% Total
1 R1	2487	100.00	82.90
2 R2	2487	100.00	82.90
3 R3	58	2.33	1.93



224FL001\FL1-H-FL1-Height

Arithmetic Histogram Statistics for 224FL001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= RIANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2487	100.00	32	5.23
1	1.00, 2.01	151	6.07	12	2.57
2	2.01, 9.02	2253	90.59	32	5.28
3	9.02, 136	97	3.90	0	11.14

THE UNIVERSITY OF CHICAGO
ANIMAL RESOURCES CENTER

REQUEST FOR TRANSFER OF ANIMAL OWNERSHIP

Requested transfer date 4/18/95

We request the following animals be transferred:

SPECIES jude mice

QUANTITY 56

CAGE CARD NUMBER(S)

AA113928 through AA113943 (16 cages)

REQUESTED TRANSFER FROM:

PRINCIPAL INVESTIGATOR _____

PROTOCOL _____ FAS ACCOUNT _____

PHONE _____ SIGNATURE _____

☐ this animal(s) been used in research or teaching? YES NO

If yes how? _____

The number of unused animals transferred will be restored to the number available for ordering under this ACUP.

REQUESTED TRANSFER TO:

PRINCIPAL INVESTIGATOR HAWKMAN / SIBLER

PROTOCOL _____ FAS ACCOUNT _____

PHONE 2-0294 SIGNATURE [Signature]

Will this animal(s) be moved to a new housing site / room? ☒ YES NO

If yes where? CARLSON J-013

The number of animals transferred will be deducted from the number available for ordering under this ACUP.

ARC APPROVALS:

CLINICAL VETERINARIAN APPROVAL _____ DATE _____

Animals which have been used or which are to be moved to a different housing site require the approval of a clinical veterinarian.

BUSINESS OFFICE APPROVAL _____ DATE _____

ANSACTION DATE _____

GCS ENTRY BY _____

ACUP No.: 58671B

ACUP Amendment-Supplemental Form A

Only the primary investigator of an ACUP is permitted to make changes to that ACUP. Please note that certain changes in an ACUP may affect other aspects of that ACUP, and should be reflected in this amendment. ACUPs with amendments require Institutional Animal Care and Use Committee (IACUC) review and approval. The IACUC reserves the right to determine whether proposed changes are substantive or not, and to request further information or a new ACUP application, as appropriate. When submitting an amendment, the Principal Investigator is required to review all of the details of the original ACUP and to assure the IACUC that all unamended details remain identical to the original ACUP. Please note that the amendment must be typed.

Investigator: Dennis Hallahan, M.D.

Department: Radiation Oncology

ACUP Number: 58671B

Original approval date:

The following changes are herein proposed for this protocol:

☐ staff involved

Note: Include the number of years of work experience with the species used in this protocol, and confirm that each individual has reviewed the University of Chicago Manual on Laboratory Animals Interim Guide. If anyone listed has less than one year of relevant experience, please briefly describe how they will be or have been trained.

☐ housing ☒ procedure ☐ agents used☐ number of animals ☐ change in co-investigator☐ site outside centrally managed animal facilities to which live animals are taken and route of animal transport☒ response to questions raised during the IACUC-ARC review process ☐ other

Provide sufficient details, including a rationale for the proposed change, in narrative form to allow the committee to make a decision. Use additional pages as necessary and submit revised pages of the original ACUP, as appropriate.

Clarification of endpoints used in protocol #58671B:

1.) Tumor Size: The cutoff as mentioned in the protocol is "10% of body weight". Tumor measurements are typically performed twice weekly and tumor volumes are estimated by the formula $1/2(\text{length} \times \text{width} \times \text{height})$. This is derived from the equation for the volume of a sphere $= 4\pi r^3/3 = 4r^3/3 = d^3/2 = (1 \times w \times h)/2$. Since most nude mice weigh 18-25 grams, a tumor size of $2000 \text{ mm}^3 = 2 \text{ cc} = 2 \text{ grams}$ is approximately 10% of body weight. This will be used as the cutoff for tumor size and can be easily verified by those monitoring the animals.

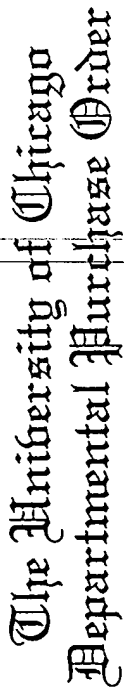
2.) Ulceration of tumors: When rapidly growing tumors outgrow their blood supply, they typically undergo necrosis in the central portion of the tumor. This creates a necrotic cavity which may heal completely over time if the tumor is cured. This situation is also seen in humans, most commonly in patients undergoing treatment for head and neck cancers. In our experience, this central necrosis is not tender in nude mice and has not predisposed the animals to infection, weight loss or loss of limb function. We continue to use these latter three conditions as criteria to sacrifice the animals.



Investigator

This form must be submitted bearing the original signature of the investigator.

4/18/95
Date



Purchase Order Number

Z890627

THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

VWR Scientific
P.O. Box 66929
O'Hare AMF
Chicago
IL 60666
State Zip Code
Delivery charge? ☐ Yes ☒ NO
Telephone No. 1-800-432-5000 FAX No.

Payment Terms _____ Delivery charge? ☐ Yes ☒ No
 Telephone No. 1-800-932-5000 FAX No. 1-800-932-5000

THE UNIVERSITY OF CHICAGO

Radiation Oncology Department Oncology 5830 S. Ellis Ave Chicago Ill	6-03 Room	1C 60637 State Zip Code
---	--------------	---------------------------------

Authorized Signature

Payroll No.

Print Name--

Exl

Account Code

Date _____

Order placed by phone?

☒ Yes
☐ No

Sharon	4/24/55
--------	---------

Order placed with (name)	Date
	1/4/0

580-2 2-0784

Order placed by (name)	Phone #
Ernest Jones	

QTY	UNIT
-----	------

UNIT

DESCRIPTION

NET UNIT PRICE

ITEM TOTAL

4	1	Vernier Calipers
---	---	------------------

750-55161

49.88

199.52

VWR Act # 77055-172

ARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

**BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER**

THE UNIVERSITY OF CHICAGO

ANIMAL RESOURCES CENTER

ANIMAL PROCUREMENT

QUEST (#315B)

REQ NO 24129

ARC USE ONLY

P.O. #

ORDER DATE

REF #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: SHIPMENTS ON A BASISREQUEST BY: Greg Sibley, MD DATE: 4/26/95REQUESTORS PHONE NUMBER: 2-0294AUTHORIZED SIGNATURE: [Signature]FAS ACCOUNT: 2-73731-5100 PI: HallgrenVENDOR: FCRI PROTOCOL: 5861REQUESTED DELIVERY DATE: 5/3/95 PHONE: 2-0294SPECIES: Mouse QUANTITY: 80STRAIN: Albino Nude SEX: M ☒ F EITHERWEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

Mice to go to Carlson BiohazardSwite Room J-013

PROCUREMENT DESK: 2-9364

HOUSE AT: ☒ CARLSON ☐ WYLER ☒ CLSC ☐ FMI ☐ OTHER

J-013

5/1/95

Apoptosis Assay - V87 cells

Purpose - To see if apoptosis is seen in virally treated groups at earlier timepoints and to see if apoptosis is seen with TNF+RT at other time points

Methods - T-150 flasks of V87 cells were grown to ~80% confluence

			7 AM	1 PM	5 PM	7 PM	5 PM	5 PM	5 PM	%A
			-10	-4	0°	6°	24°	48°	72°	
Control								Harvest		1.8
2	TNF+RT	6°		TNF	RT	Harvest				2.6
3	"	24°		TNF	RT		Harvest			10.1
4		48°		TNF	RT			Harvest		5
5		72°		TNF	RT				Harvest	16.1
6	10^7 899-6	6°	infect			Harvest				4
10		24°	infect				Harvest			13.1
7	10^7 899-6+RT	6°	infect		RT	Harvest				5.6
11		24°	infect		RT		Harvest			25
8	10^7 899-6	6°	infect			Harvest				13.7
12		24°	infect				Harvest			11.1
9	10^7 899-6+RT	6°	infect		RT	Harvest				8.1
13		24°	infect		RT		Harvest			9

Infection - cells were infected in 6 ml of 199V med'g x 2° then additional 14 cc of 10 FCS med'g was added

RT - single fraction of 20 Cg

TNF - 10 ul of 0.01 mg/ml stock in 20 x 1 med'g

Control
+ TdT

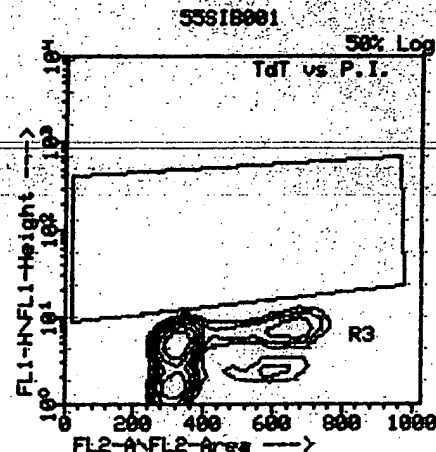
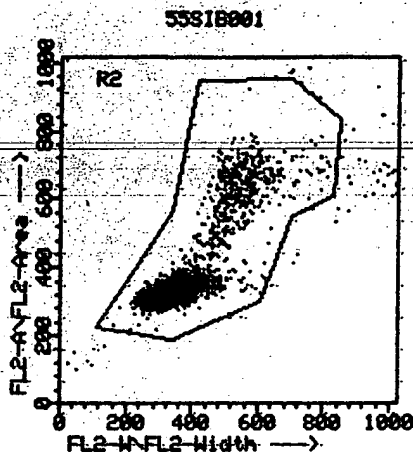
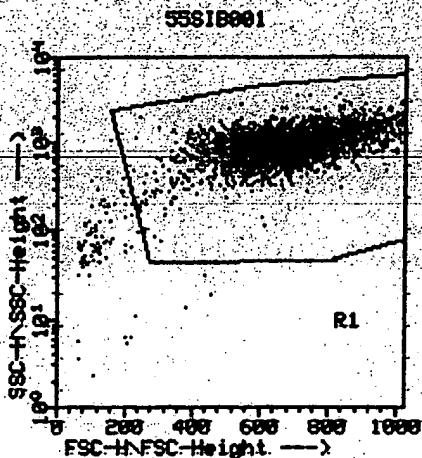
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:04:24

SELECTED PREFERENCES: Arithmetic/Linear



55SIB001

----- Region Stats -----

File: 55SIB001 Sample: SIBLEY APOP :

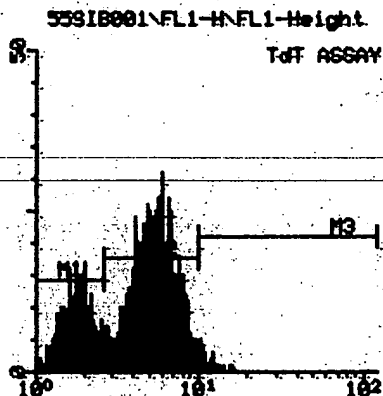
Date: 5/ 5/95 Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Line

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total
1 R1	2399	100.00	79.97
2 R2	2399	100.00	79.97
3 R3	1	0.04	0.03



55SIB001\FL1-H\FL1-Height

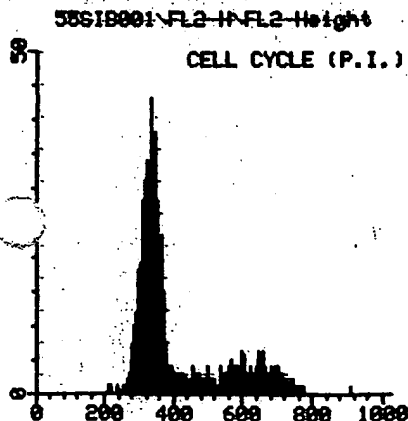
----- Arithmetic Histogram Statistics for 55SIB001

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M Left,Right Events % Peak Median

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2399	100.00	31	4.74
1	1.00, 2.64	646	26.93	17	1.70
2	2.64, 9.91	1717	71.57	31	5.42
3	9.91, 110	44	1.83	5	10.89



1.8

TNF-RT 6°

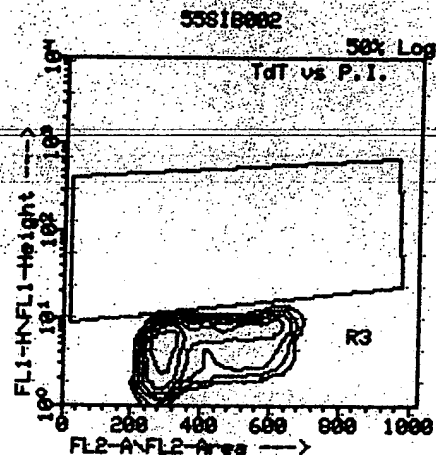
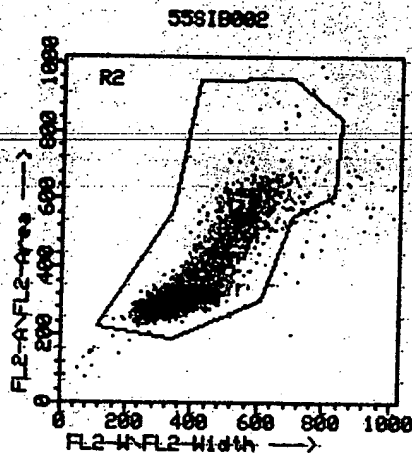
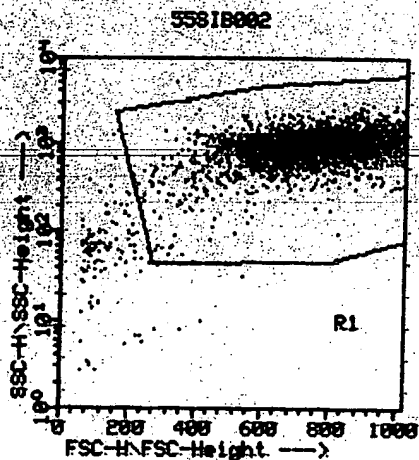
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:05:22

SELECTED PREFERENCES: Arithmetic/Linear

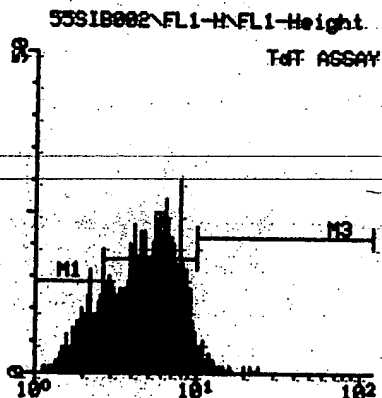


55818002

Region Stats

File: 55818002 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate 02= R1ANDR2
Selected Preferences: Arithmetic/Linear
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2450

Rgn	Events	% Gated	% Total	
1 R1	2450	100.00	81.67	1:
2 R2	2450	100.00	81.67	1:
3 R3	4	0.16	0.13	1:

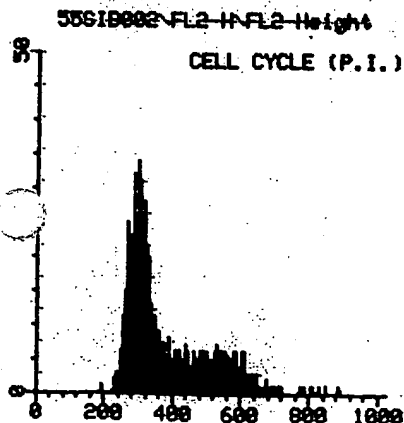


55818002-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55818002

Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate 02= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2450	100.00	38	4.83
1	1.00, 2.64	400	16.33	16	2.15
2	2.64, 9.91	2003	81.76	30	5.28
3	9.91, 110	63	2.57	5	10.84



2.6

INERT 480

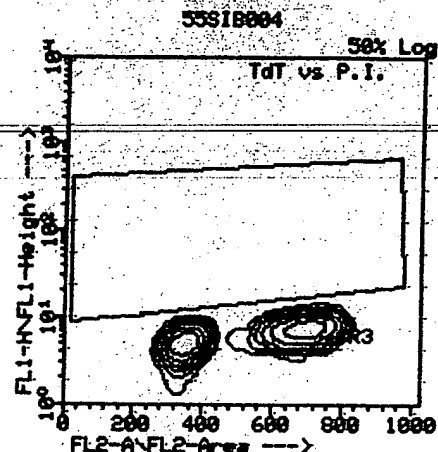
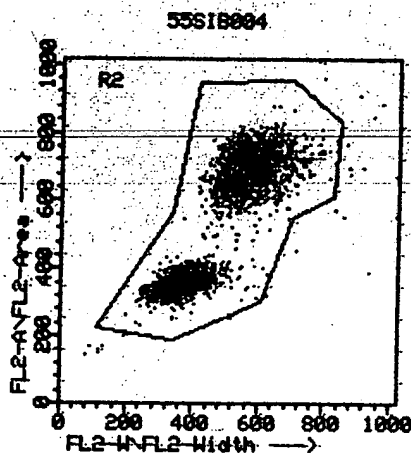
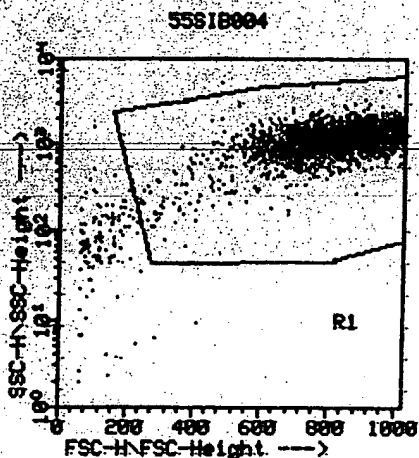
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:07:20

SELECTED PREFERENCES: Arithmetic/Linear



55SIB004

Region Stats

File: 55SIB004 Sample: SIBLEY APOP :

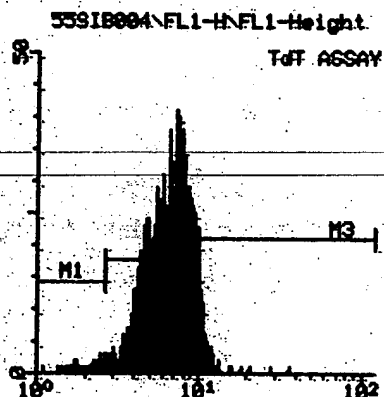
Date: 5/ 5/95 Gate: G2= R1ANDR2

Selected Preferences: Arithmetic/Line.

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2479

Rgn	Events	% Gated	% Total
1 R1	2479	100.00	82.63
2 R2	2479	100.00	82.63
3 R3	4	0.16	0.13



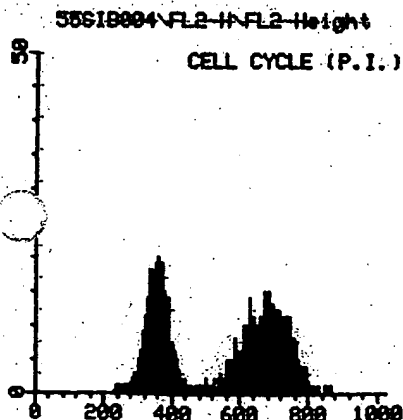
55SIB004-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55SIB004

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

N	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2479	100.00	41	6.61
1	1.00, 2.64	35	41	3	2.09
2	2.64, 9.91	23	83	41	6.53
3	9.91, 110	1	04	13	10.46



5

INERT 72°

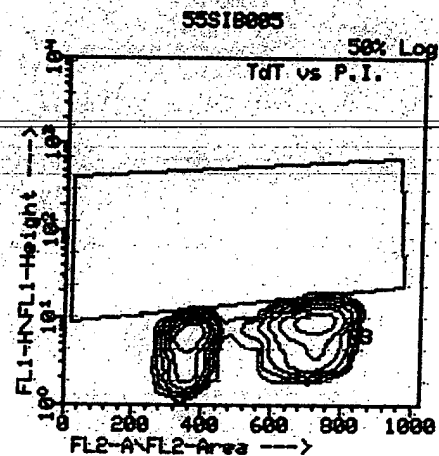
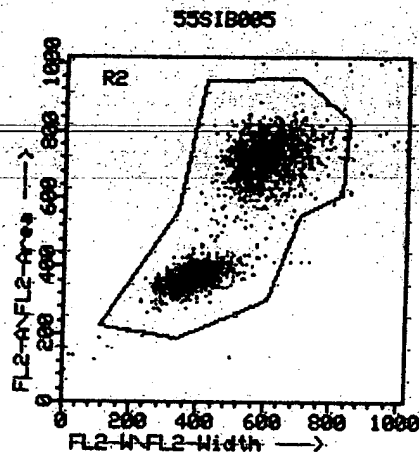
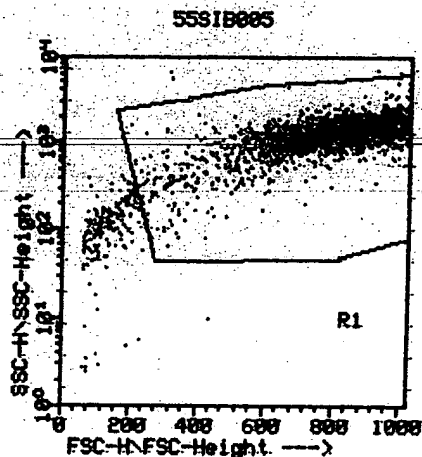
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:08:20

SELECTED PREFERENCES: Arithmetic/Linear



55SIB005

Region Stats

File: 55SIB005 Sample: SIBLEY APOP !

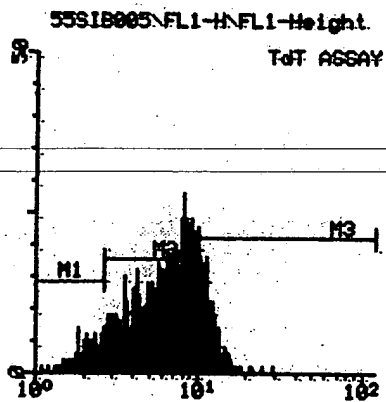
Date: 5/5/95 Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 2162

Rgn	Events	% Gated	% Total
1 R1	2162	100.00	72.07
2 R2	2162	100.00	72.07
3 R3	7	0.32	0.23



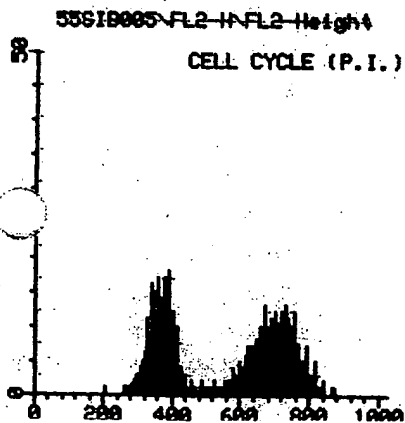
55SIB005 FL1-H-FL1-Height

Arithmetic Histogram Statistics for 55SIB005

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

H	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2162	100.00	28	6.73
1	1.00, 2.64	167	7.72	7	2.19
2	2.64, 9.91	1636	76.60	28	6.38
3	9.91, 110	360	16.65	18	11.14



16.7

BECTON
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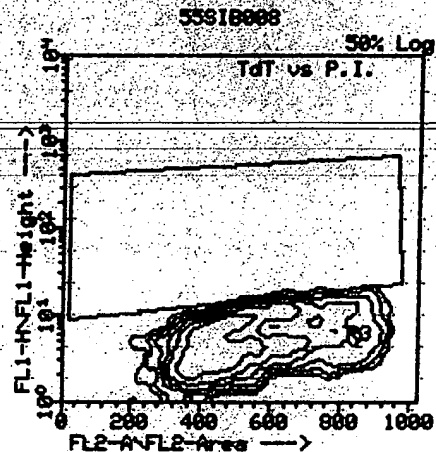
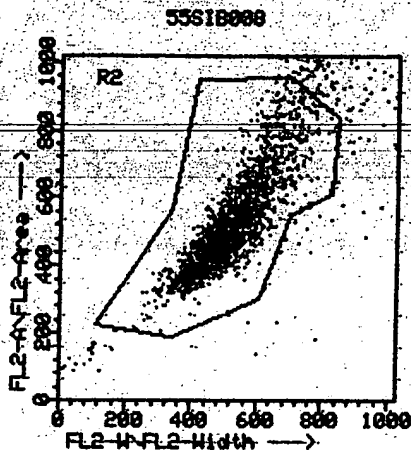
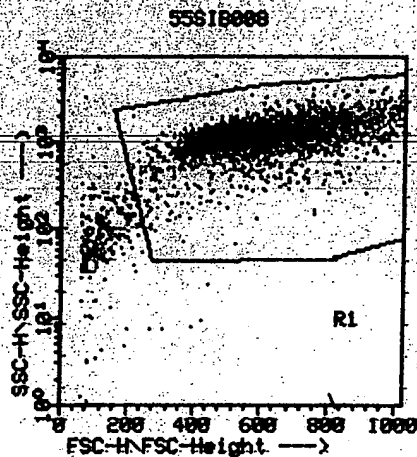
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:11:13

SELECTED PREFERENCES: Arithmetic/Linear

7
2x10⁸ 89A-6-6°

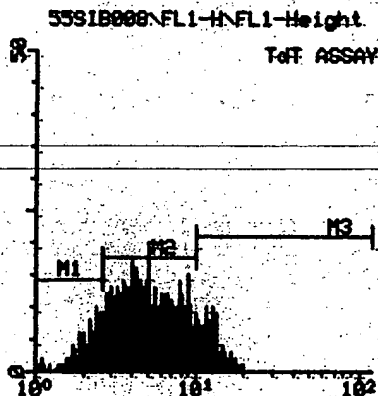


55SIB008

Region Stats

File: 55SIB008 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1748
Rgn Events % Gated % Total :

Rgn	Events	% Gated	% Total	:
1 R1	1748	100.00	58.27	1.
2 R2	1748	100.00	58.27	1.
3 R3	2	0.11	0.07	1

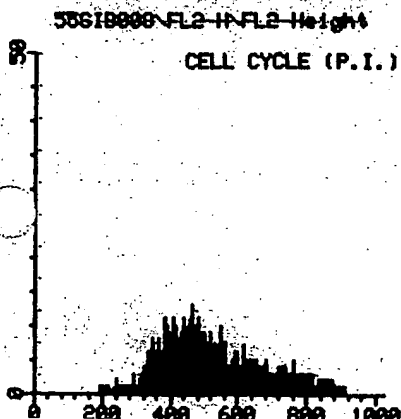


55SIB008\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB008

Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2
M Left,Right Events % Peak Median

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	20	4.78
1	1.00, 2.64	225	12.87	10	2.23
2	2.64, 9.91	1298	74.26	20	4.74
3	9.91, 118	240	13.73	10	11.92



13.7

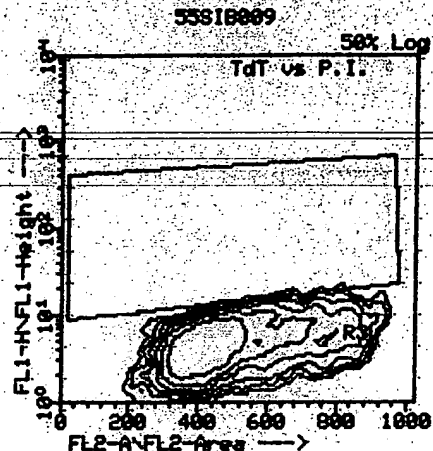
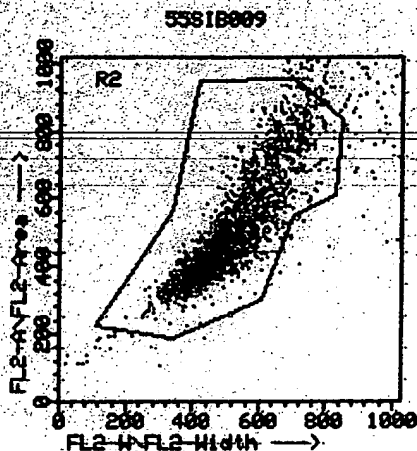
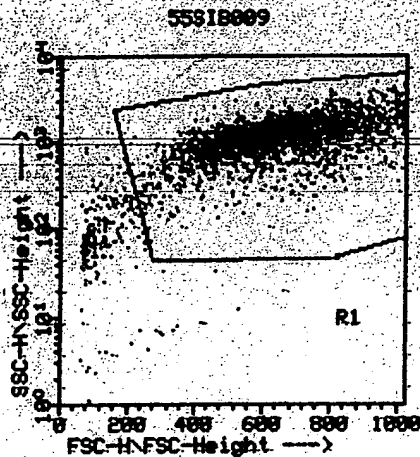
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:12:14

SELECTED PREFERENCES: Arithmetic/Linear



55818009

Region Stats

File: 55818009 Sample: SIBLEY APOP !

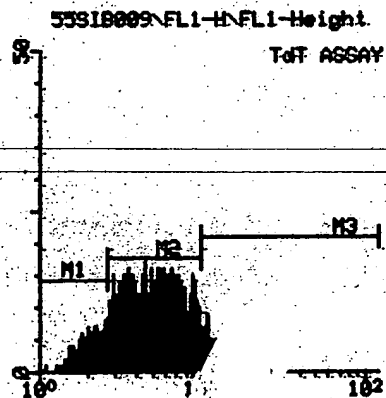
Date: 5/5/95 Gate G2= R1ANDR2

Selected Preference: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LDG)

Total= 3000 Gated= 1817

Rgn	Events	% Gated	% Total	
1 R1	1817	100.00	60.57	1:
2 R2	1817	100.00	60.57	1:
3 R3	6	0.33	0.20	1:



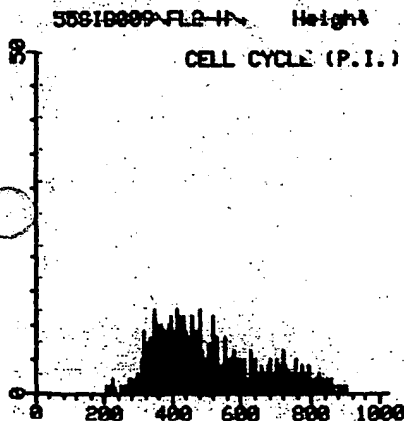
55818009 FL1-H FL1-Height

Arithmetic Histogram Statistics for 55818009

Selected Preference: Arithmetic/Linear

Parameter: FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1817	100.00	17	5.00
1	1.00, 2.64	236	12.99	8	2.13
2	2.64, 9.91	1435	78.98	17	5.19
3	9.91, 118	156	8.59	9	11.44



8.6

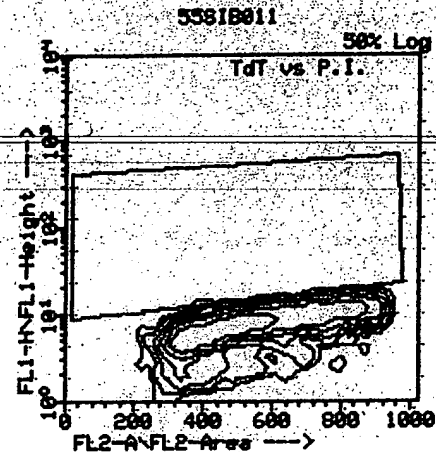
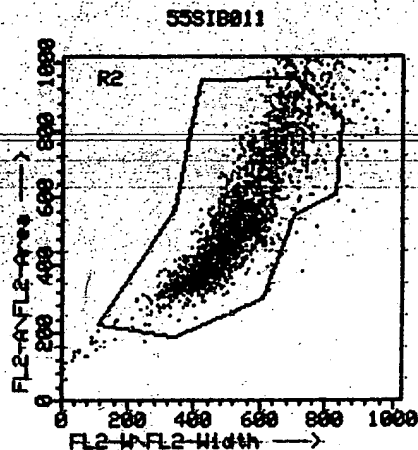
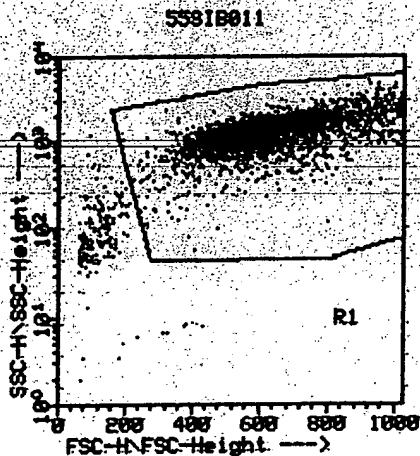
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:14:11

SELECTED PREFERENCES: Arithmetic/Linear

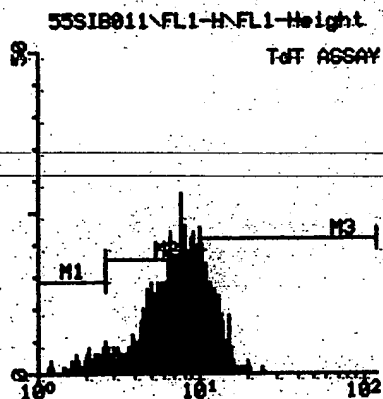


55SIB011

Region Stats

File: 55SIB011 Sampler: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1815

Rgn	Events	% Gated	% Total
1 R1	1815	100.00	60.50
2 R2	1815	100.00	60.50
3 R3	5	0.28	0.17



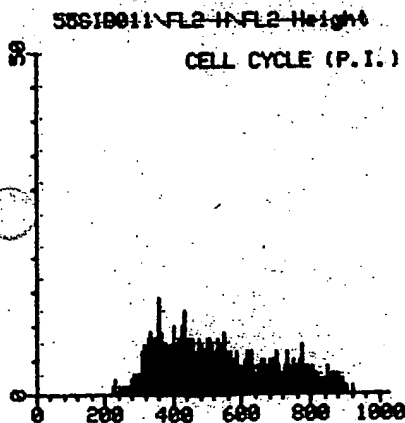
55SIB011\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55SIB011

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 99.10	1815	100.00	28	7.64
1	1.00, 2.64	95	5.23	5	2.09
2	2.64, 9.91	1290	71.07	28	6.92
3	9.91, 110	454	25.01	28	11.53



25

2x10⁷ 886-6 24°

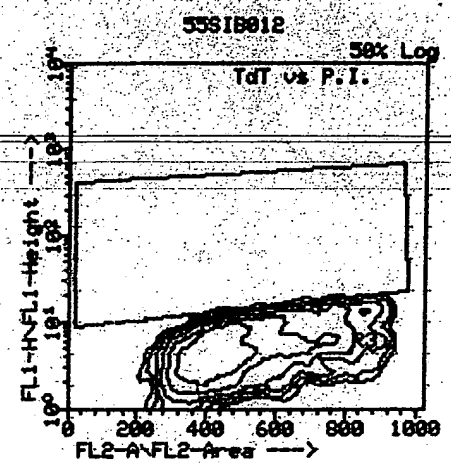
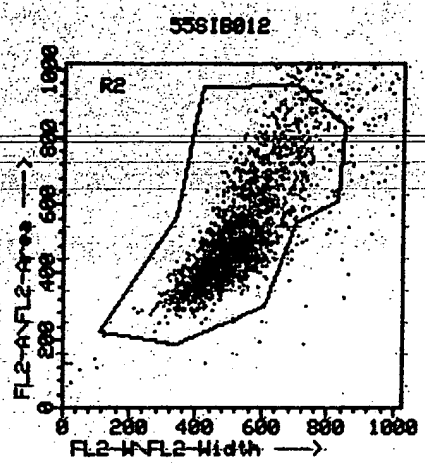
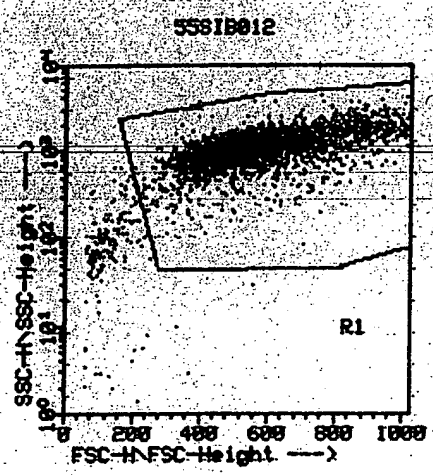
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:15:11

SELECTED PREFERENCES: Arithmetic/Linear



5581B012

Region Stats

File: 5581B012 Sample: SIBLEY APOP !

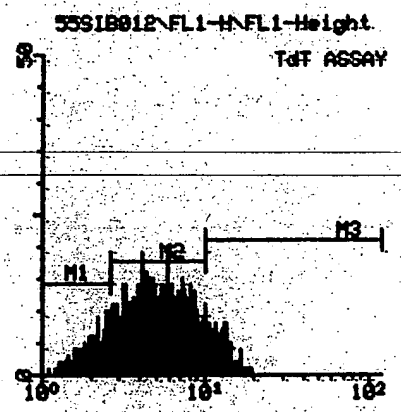
Date: 5/ 5/95 Gate G2= R1ANDR2

Selected Preferences: Arithmetic/Linear

Parameters: FL2-A(LIN), FL1-H(LOG)

Total= 3000 Gated= 1795

Rgn	Events	% Gated	% Total	
1 R1	1795	100.00	59.83	1:
2 R2	1795	100.00	59.83	1:
3 R3	3	0.17	0.10	1:



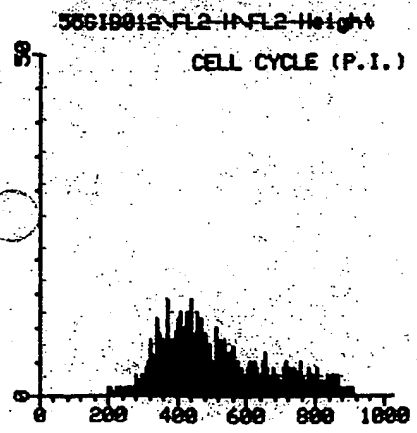
5581B012-FL1-H-FL1-Height

Arithmetic Histogram Statistics for 5581B012

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1795	100.00	19	4.91
1	1.00, 2.64	269	14.99	10	2.11
2	2.64, 9.91	1339	74.60	19	5.09
3	9.91, 110	205	11.42	11	11.65



11.4

2x10⁷ 8946+RT 24

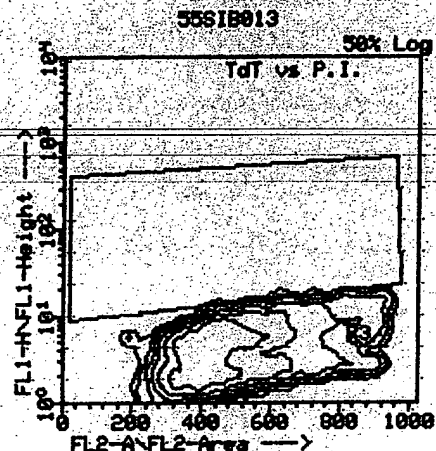
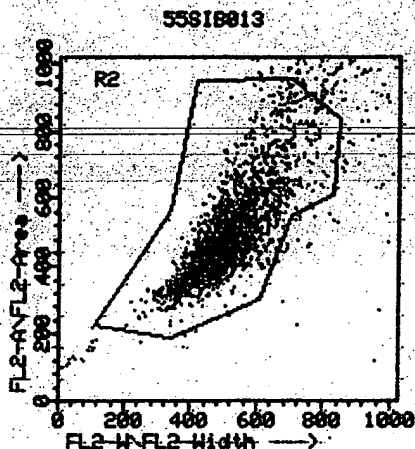
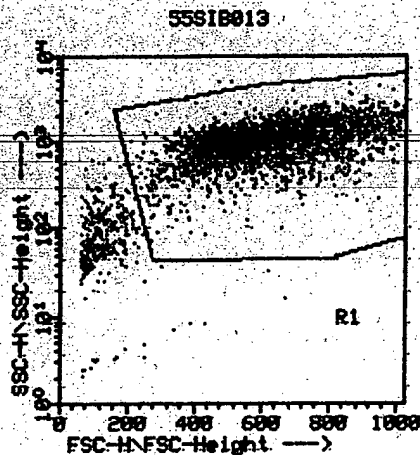
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:16:10

SELECTED PREFERENCES: Arithmetic/Linear

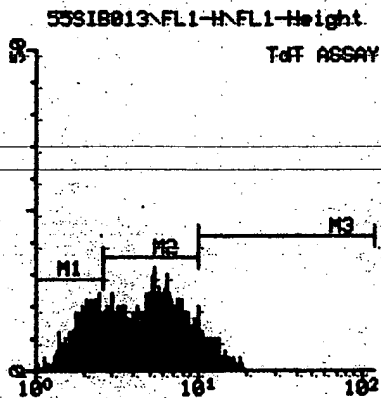


55SIB013

----- Region Stats -----

File: 55SIB013 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Linear
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 1622

Rgn	Events	% Gated	% Total	
1 R1	1622	100.00	54.07	1
2 R2	1622	100.00	54.07	1
3 R3	4	0.25	0.13	1

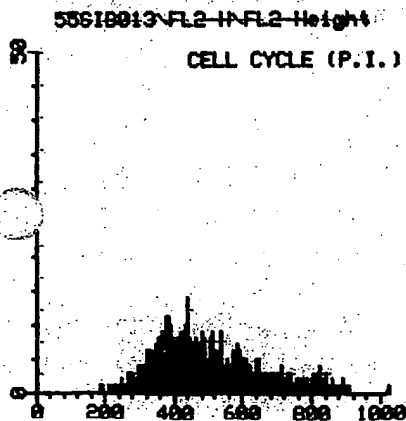


55SIB013-FL1-H-FL1-Height

----- Arithmetic Histogram Statistics for 55SIB013 -----

Selected Preferences: Arithmetic/Linear
Parameter: FL1-H-FL1-Height Gate G2= R1ANDR2

H	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1622	100.00	16	4.49
1	1.00, 2.64	416	25.65	12	2.07
2	2.64, 9.91	1074	66.21	16	3.19
3	9.91, 110	148	9.00	10	11.06



9

Pos Control
OT

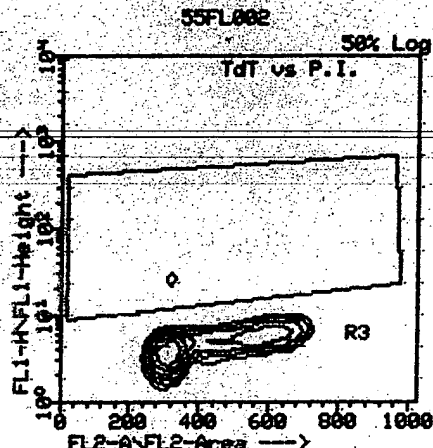
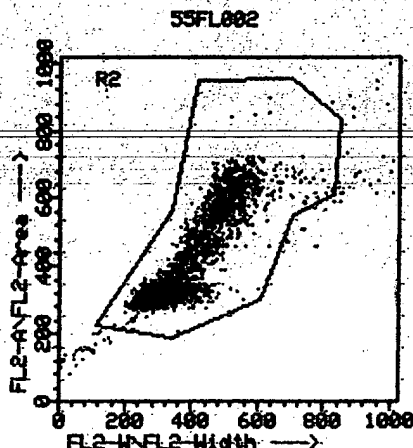
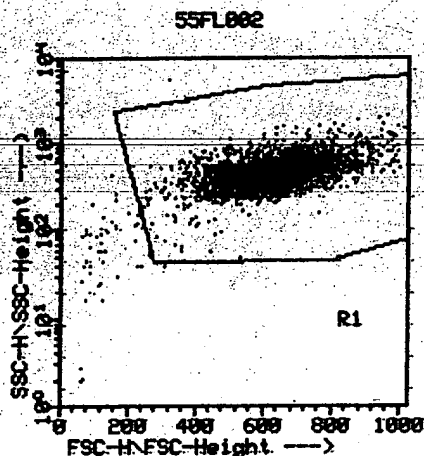
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:19:50

SELECTED PREFERENCES: Arithmetic/Linear

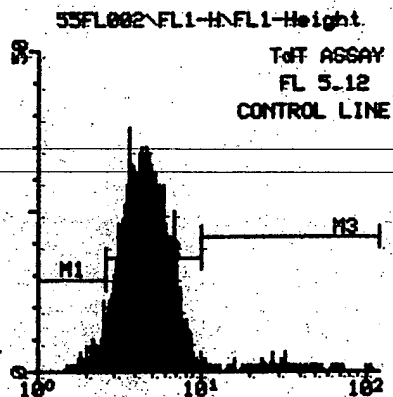


55FL002

Region Stats

File: 55FL002 Sample: SIBLEY APOP 5
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line.
Parameters: FL2-A(LIN), FL1-H(LOG)
Total= 3000 Gated= 2684

Rgn	Events	% Gated	% Total
1 R1	2684	100.00	89.47
2 R2	2684	100.00	89.47
3 R3	58	2.16	1.93

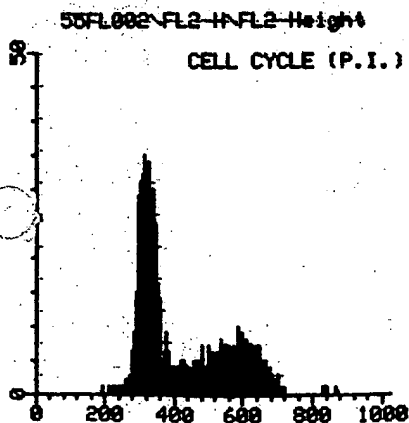


55FL002\FL1-H\FL1-Height

Arithmetic Histogram Statistics for 55FL002

Selected Preferences: Arithmetic/Linear
Parameter: FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9918	2684	100.00	38	4.49
1	1.00, 2.64	121	4.51	11	2.35
2	2.64, 9.91	2581	93.18	38	4.53
3	9.91, 118	68	2.53	3	27.14



2.5

Positive Control
⊕ Td Control

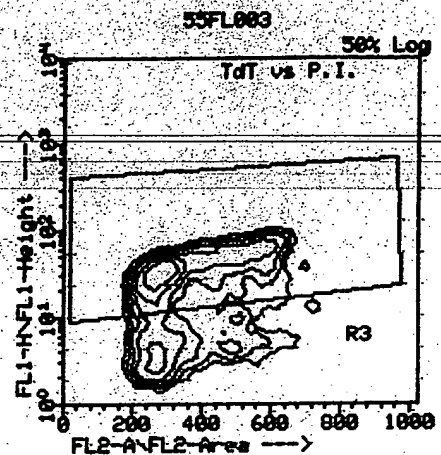
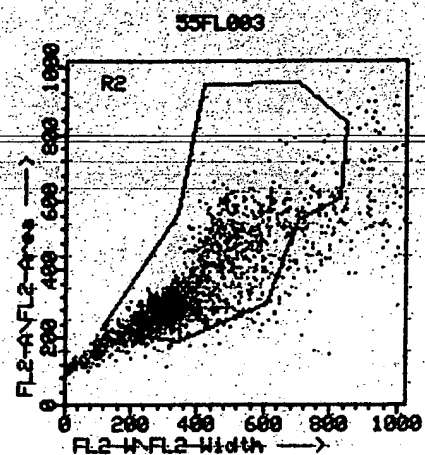
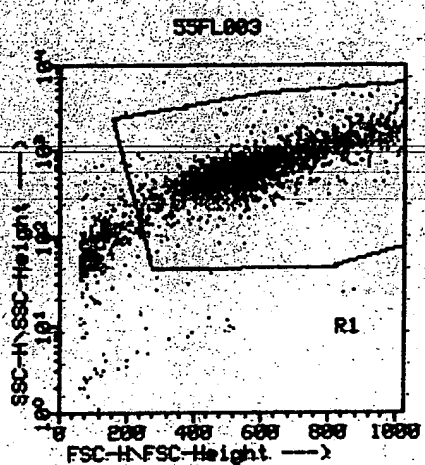
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LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

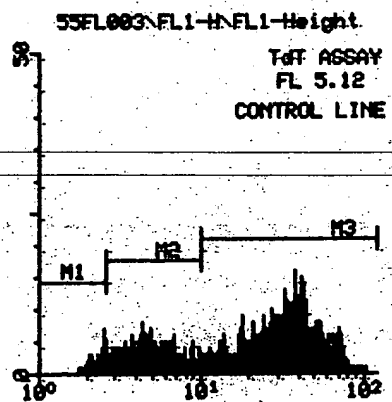
TIME: 15:21:40

SELECTED PREFERENCES: Arithmetic/Linear



55FL003
 Region Stats
 File: 55FL003 Sample: SIBLEY APOC 5.
 Date: 5/ 5/95 Gate G2= R1ANDR2
 Selected Preferences: Arithmetic/Linear
 Parameters: FL2-A(LIN), FL1-H(LOG)
 Total= 3000 Gated= 1439

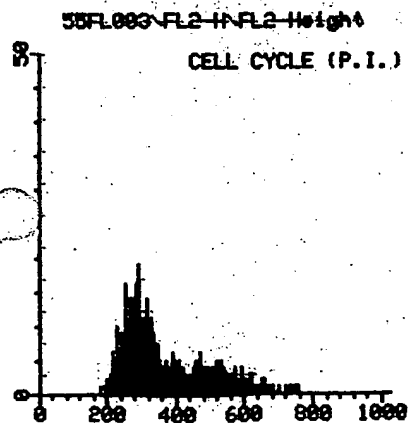
Rgn	Events	% Gated	% Total
1 R1	1439	100.00	47.97
2 R2	1439	100.00	47.97
3 R3	962	66.85	32.07



55FL003-FL1-H-FL1-Height
 Arithmetic Histogram Statistics for 55FL003
 Selected Preferences: Arithmetic/Linear
 Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1439	100.00	16	25.71
1	1.00, 2.64	36	3.89	7	2.44
2	2.64, 9.91	374	25.99	8	4.96
3	9.91, 110	1012	70.33	16	34.29

70.3



5/4/95

Mouse #	Cage #	Group	Day 0			weight	Day 3			weight
1710	AA105283	10^7 899-6	0	0	0					
2075	AA105283	10^7 899-6	0	0	0					
1713	AA105283	10^7 899-6	0	0	0					
2054	AA111331	10^7 899-6	0	0	0					
2057	AA111332	10^7 899-6								
1709	AA104549	10^7 3616								
1723	AA104549	10^7 3616	0	0	0					
2041	AA111334	10^7 3616								
2064	AA111334	10^7 3616								
1859	AA108972	RT Alone	12.5	11.5	9					
1861	AA108972	RT Alone								
1862	AA108972	RT Alone	6	5	4					
1892	AA108964	RT Alone	6	4.5	3					
1863	AA108971	10^7 3616+RT	-4.5	4	3.5					
1864	AA108971	10^7 3616+RT	-14	9.5	7					
1865	AA108971	10^7 3616+RT	-4.5	3.5	1					
1866	AA108971	10^7 3616+RT	-4	5	1					
1883	AA108966	10^7 3616+RT	-0	0	0					
1884	AA108966	10^7 3616+RT	-1	1	1					
1886	AA108966	10^7 3616+RT	-0	0	0					
2074	AA108966	10^7 3616+RT	-9.5	8.5	6					
2032	AA111326	10^7 3616+RT	-6	5	4.5					
2033	AA111326	10^7 3616+RT	-5	4	3					
2035	AA111326	10^7 3616+RT	-8	7	4.5					
2072	AA111326	10^7 3616+RT	0	0	0					
2036	AA111327	10^7 3616+RT	-7	6	3.5					
2037	AA111327	10^7 3616+RT	4	3	1.5					
2039	AA111327	10^7 3616+RT	9	8	5					
2073	AA111327	10^7 3616+RT	-4	5	1.5					
1871	AA108969	10^7 899-6+RT	3.5	3.5	2					
1872	AA108969	10^7 899-6+RT								
1873	AA108969	10^7 899-6+RT	-5	7	4.5					
1874	AA108969	10^7 899-6+RT	-0	0	0					
1880	AA108967	10^7 899-6+RT	-0	0	0					
1881	AA108967	10^7 899-6+RT	0	0	0					
18	AA108967	10^7 899-6+RT	2.5	2	1					
20	AA111329	10^7 899-6+RT	4.5	3.5	2.5					
2046	AA111329	10^7 899-6+RT	0	0	0					
2047	AA111329	10^7 899-6+RT	4.5	5	2.5					
2048	AA111330	10^7 899-6+RT	-2.5	2.5	1.5					
2049	AA111330	10^7 899-6+RT	16	13.5	11.5					
2050	AA111330	10^7 899-6+RT	2.5	3.5	2					
2051	AA111330	10^7 899-6+RT	9	8	4.5					

[illegible]

LOG SHEETS

5/8

1710	AA105283	10^7 899-6	<10 d35	0	23.7		
2075	AA105283	10^7 899-6	<10 d42	0	23.0		
1713	AA105283	10^7 899-6	<10 d14	0	22.2		
2054	AA111331	10^7 899-6	<10 d49	0	23.4		
1709	AA104549	10^7 3616	<10 d31				
1723				0	24.3		
1859	AA108972	RT Alone		12.5x11x10	24.0		
1862	AA108972	RT Alone		5.5x3	25.1		
1892	AA108964	RT Alone		5x4x2	24.4		
2189	AA113941	RT Alone		10x11.5x8	22.0		
2190	AA113941	RT Alone		11.5x9.5x7	18.2		
2191	AA113941	RT Alone		12.5x8x6.5	18.6		
2192	AA113941	RT Alone		12x10x8	22.0		
2201	AA113944	RT Alone		8.5x10x8	22.6		
2202	AA113944	RT Alone		8.5x10x7	21.3		
2203	AA113944	RT Alone		8x7x6	24.3		
2204	AA113944	RT Alone		12.5x12.5x10	23.1		
1863	AA108971	10^7 3616+RT		5.5x4x3	24.7		
1864	AA108971	10^7 3616+RT		13.5x10x6.5	27.6		
1865	AA108971	10^7 3616+RT	<10 d38	4x3x1	27.0		
1866	AA108971	10^7 3616+RT	<10 d31	0	26.3		
1883	AA108966	10^7 3616+RT	<10 d24	0	22.6		
1884	AA108966	10^7 3616+RT	<10 d56	2x2x1.5	23.9		
1886	AA108966	10^7 3616+RT	<10 d31	0	21.5		
2074	AA108966	10^7 3616+RT		9.5x9x6	17.0		
2032	AA111326	10^7 3616+RT		6x7.5x4.5	22.1		
2033	AA111326	10^7 3616+RT		4x4x2.5	25.0		
2035	AA111326	10^7 3616+RT		7x8.5x5	22.2		
2072	AA111326	10^7 3616+RT	<10 d21	0	24.7		
2036	AA111327	10^7 3616+RT		6x2.5x4.5	22.1		
2037	AA111327	10^7 3616+RT	<10 d52	3x2x1	25.3		
2039	AA111327	10^7 3616+RT		8.5x9x5.5	17.6		
2073	AA111327	10^7 3616+RT		4x4x1	20.6		
				6.5x6x3	22.8		
1871	AA108969	10^7 899-6+RT	<10 d66	3.5x3.5x2	24.0		
1873	AA108969	10^7 899-6+RT		6x6x4	24.6		
1874	AA108969	10^7 899-6+RT	<10 d52	0	27.8		
1880	AA108967	10^7 899-6+RT	<10 d14	0	21.7		
1881	AA108967	10^7 899-6+RT	<10 d42	0	26.4		
1882	AA108967	10^7 899-6+RT	<10 d24	0	24.0		
2044	AA111329	10^7 899-6+RT		4.5x4x3	23.8		
2045	AA111329	10^7 899-6+RT	<10 d21	0	27.3		
2046	AA111329	10^7 899-6+RT		4.5x4x2	25.0		
2048	AA111330	10^7 899-6+RT	<10 d38	2x2.5x1	15.9		
2049	AA111330	10^7 899-6+RT		18.5x4x3.5	25.2		
2050	AA111330	10^7 899-6+RT	<10 d31	3x2x1.5	23.9		
2051	AA111330	10^7 899-6+RT		7x8x3.5	24.0		

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1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1723				0			
1859	AA108972	RT Alone	11.5 x 10 x 10.5				
1862	AA108972	RT Alone	4.5 x 5 x 3.5				
1892	AA108964	RT Alone	4.5 x 3.5 x 3				
2189	AA113941	RT Alone	10.5 x 11 x 9				
2190	AA113941	RT Alone	11.5 x 8 x 8.5				
2191	AA113941	RT Alone	10.5 x 7 x 6.5				
2192	AA113941	RT Alone	11 x 12 x 9				
2201	AA113944	RT Alone	10 x 9.5 x 9				
2202	AA113944	RT Alone	7.5 x 7 x 6.5				
2203	AA113944	RT Alone	8.5 x 10 x 7				
2204	AA113944	RT Alone	11.5 x 11 x 9				
1863	AA108971	10^7 3616+RT		4 x 5 x 3			
1864	AA108971	10^7 3616+RT		15 x 12 x 8			
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	2 x 2 x 1.5			
1886	AA108966	10^7 3616+RT	<10 d31	3 x 4 x 2.5 0			
2074	AA108966	10^7 3616+RT		8 x 9 x 4			
2032	AA111326	10^7 3616+RT	5 x 7 x 4.5	5 x 7 x 4.5			
2033	AA111326	10^7 3616+RT		4 x 4 x 2.5			
2035	AA111326	10^7 3616+RT		8 x 6 x 5			
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		5.5 x 6.5 x 3			
2037	AA111327	10^7 3616+RT	<10 d52	3 x 2.5 x 1.5			
2039	AA111327	10^7 3616+RT		10 x 8 x 6			
2073	AA111327	10^7 3616+RT		4 x 3 x 1			
1871	AA108969	10^7 899-6+RT	<10 d66	3 x 4 x 2.5			
1873	AA108969	10^7 899-6+RT		6 x 5 x 3			
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		5 x 4 x 4			
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		4 x 3.5 x 2.5			
2048	AA111330	10^7 899-6+RT	<10 d38	1.5 x 1 x 1			
2049	AA111330	10^7 899-6+RT		18 x 13 x 11			
2050	AA111330	10^7 899-6+RT	<10 d31	3 x 2 x 1			
2051	AA111330	10^7 899-6+RT		6 x 8 x 4.5			

OVER

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1710	AA105283	10^7 899-6	<10 d35	0	0	0	23.8
2075	AA105283	10^7 899-6	<10 d42	0	0	0	22
1713	AA105283	10^7 899-6	<10 d14	0	0	0	20.8
2054	AA111331	10^7 899-6	<10 d49	0	0	0	22.4
1709	AA104549	10^7 3616	<10 d31	0	0	0	25.3
23							
1859	AA108972	RT Alone		12	14	10	24.5
1862	AA108972	RT Alone		5.5	9	3	24.4
1892	AA108964	RT Alone		5	5	3	23.5
2189	AA113941	RT Alone		11	12	8	22.0
2190	AA113941	RT Alone		12	9.5	7	17.8
2191	AA113941	RT Alone		12	8.5	6	19.2
2192	AA113941	RT Alone		12	11	9	20.3
2201	AA113944	RT Alone		11	12	9	22.2
2202	AA113944	RT Alone		6.5	6.5	5.5	24.2
2203	AA113944	RT Alone		11.5	10	8.5	20.4
2204	AA113944	RT Alone		12	11.5	7	19.9
1863	AA108971	10^7 3616+RT		5	4	2	23.3
1864	AA108971	10^7 3616+RT		14	13	7	25.4
1865	AA108971	10^7 3616+RT	<10 d38	0	0	0	25.6
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	25.2
1883	AA108966	10^7 3616+RT	<10 d24	0	0	0	22.1
1884	AA108966	10^7 3616+RT	<10 d56	0	0	0	23.9
1886	AA108966	10^7 3616+RT	<10 d31	0	0	0	21.4
2074	AA108966	10^7 3616+RT		10	9.5	4	16.8
2032	AA111326	10^7 3616+RT		8	7	5.5	23.2
2033	AA111326	10^7 3616+RT		4.5	4	1.5	25.1
2035	AA111326	10^7 3616+RT		0	0	0	24.4
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	4	23.3
2036	AA111327	10^7 3616+RT		6	6.5	3	23.8
2037	AA111327	10^7 3616+RT	<10 d52	5	3.5	1.5	25.2
2039	AA111327	10^7 3616+RT		11	9	6	16.8
2073	AA111327	10^7 3616+RT		0	0	0	20.9
1871	AA108969	10^7 899-6+RT	<10 d66	7	6	2.5	23.9
1873	AA108969	10^7 899-6+RT		5	5	3	23.3
1874	AA108969	10^7 899-6+RT	<10 d52	0	0	0	27.9
1880	AA108967	10^7 899-6+RT	<10 d14	0	0	0	21.2
1881	AA108967	10^7 899-6+RT	<10 d42	0	0	0	25.1
1882	AA108967	10^7 899-6+RT	<10 d24	6	5	1.5	23.9
2044	AA111329	10^7 899-6+RT		7	6	4.5	24.3
2045	AA111329	10^7 899-6+RT	<10 d21	3.5	3	2	25.4
2046	AA111329	10^7 899-6+RT		0	0	0	25.4
2048	AA111330	10^7 899-6+RT	<10 d38	0	0	0	16.2
2049	AA111330	10^7 899-6+RT		21.5	15	14	25.3
2050	AA111330	10^7 899-6+RT	<10 d31	0	0	0	23.8
2051	AA111330	10^7 899-6+RT		8	10	5.5	24.6

113 945 L 2089 8.5 9.5 7.5 21.3
R 2090 9 8.5 7.5 22
LR

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1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
25							
1859	AA108972	RT Alone		12.5	14	10.5	
1862	AA108972	RT Alone		5	5	3.5	
1892	AA108964	RT Alone		4.5	5	2.5	
2189	AA113941	RT Alone		11	11.5	8	
2190	AA113941	RT Alone		9	12	6.5	
2191	AA113941	RT Alone		8	11	4	
2192	AA113941	RT Alone		11	11	7	
2201	AA113944	RT Alone		11	11	7	
2202	AA113944	RT Alone		7	7	5	
2203	AA113944	RT Alone		11.5	12	9	
2204	AA113944	RT Alone		12	12	8.5	
1863	AA108971	10^7 3616+RT		4	5	2	
1864	AA108971	10^7 3616+RT		14	11.5	6	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	13.0	10.0	6.0	
2074	AA108966	10^7 3616+RT		13	10	6	
2032	AA111326	10^7 3616+RT		6.5	8	6	
2033	AA111326	10^7 3616+RT		4	3.5	2	
2035	AA111326	10^7 3616+RT		0			
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	6	
2036	AA111327	10^7 3616+RT		6	7	2.5	
2037	AA111327	10^7 3616+RT	<10 d52	4	4	2	
2039	AA111327	10^7 3616+RT		12	10	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	6	5.5	2.5	
1873	AA108969	10^7 899-6+RT		5	5	3	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		6.5	6	4	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3.5	3.5	2	
2048	AA111330	10^7 899-6+RT	<10 d38	3.5	3	1	
2049	AA111330	10^7 899-6+RT		22000			
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		7.5	10	5	

113945 2206 9.5 9 7.5 controls
 2207 11 11.5 9

OVER

5/22/95

died
drown

1710	AA105283	10^7 899-6	<10 d35	0			23.2
2075	AA105283	10^7 899-6	<10 d42	0			22.3
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1859	AA108972	RT Alone		14	13.5	10	24
1862	AA108972	RT Alone		5	5	4	23.8
1892	AA108964	RT Alone		5	4	3	20.9
2189	AA113941	RT Alone		10.5	10	8	18.2
2190	AA113941	RT Alone		11	8	6.5	
2191	AA113941	RT Alone		11	9	7	22.2
2192	AA113941	RT Alone		10.5	10.5	8	20.3
2201	AA113944	RT Alone		6.5	5	4	24
2202	AA113944	RT Alone		10	10	7	20.6
2203	AA113944	RT Alone		11	11.5	8.5	21.1
2204	AA113944	RT Alone					
1863	AA108971	10^7 3616+RT		4	3	3	23.8
1864	AA108971	10^7 3616+RT		13.5	12	7	25.3
1865	AA108971	10^7 3616+RT	<10 d38	0			26.4
1866	AA108971	10^7 3616+RT	<10 d31	0			26.2
1883	AA108966	10^7 3616+RT	<10 d24	0			22.2
1884	AA108966	10^7 3616+RT	<10 d56	2	1	1	24.2
1886	AA108966	10^7 3616+RT	<10 d31	0			26.2
2074	AA108966	10^7 3616+RT		16	11	7	17
2032	AA111326	10^7 3616+RT		7.5	5.5	4.5	23.3
2033	AA111326	10^7 3616+RT		4	3	2.5	25.7
2035	AA111326	10^7 3616+RT		9.5	7	6	23.9
2072	AA111326	10^7 3616+RT	<10 d21	0			25.5
2036	AA111327	10^7 3616+RT	<10 d52	6	5	4	26.6
2037	AA111327	10^7 3616+RT		3	3	1.5	26
2039	AA111327	10^7 3616+RT		10	8.5	6	18
2073	AA111327	10^7 3616+RT		0			21.6
1871	AA108969	10^7 899-6+RT	<10 d66	5	4	2	25.4
1873	AA108969	10^7 899-6+RT		7	5	4	24.6
1874	AA108969	10^7 899-6+RT	<10 d52	0			28.3
1880	AA108967	10^7 899-6+RT	0 d14	0			22.0
1881	AA108967	10^7 899-6+P	0 d42	6	5	3.5	26.6
1882	AA108967	10^7 899-6+P	0 d24	0			24.2
2044	AA111329	10^7 899-6+R.		5	6.5	4.5	25.8
2045	AA111329	10^7 899-6+RT	<10 d21	0			16.7
2046	AA111329	10^7 899-6+RT		3.5	3	2	24.9
2048	AA111330	10^7 899-6+RT	<10 d38	0			15.9
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	5	3	2	25.6
2051	AA111330	10^7 899-6+RT		8.5	10.5	7.5	24.7

5/23/95

IN VIVO TNF PRODUCTION

1	2	3	4	5	6	7	8	9
Group		Mouse	Time	Tum weight	Vol Buffer	[TNF]	ETNF/tumor	TNF/ mg-tumor
2x10 ⁷ PRV 899-6		2370	3d	.45				
"		2371	3d	.26				
"		2372	3d	.37				
"		2373	3d	.44				
		2374						
2x10 ⁷ 899-6		2386	10d	.13				
"		2375	10d	.54				
"		2387						
"		2388	10d	.61				
"		2389	10d	.23				
Control		2386	10d	2.41				
"		2387	10d	.35				
"		2388	10d	.47				
"		2389	10d					
2x10 ⁷ 3616		2390	3d	0.44				
"		2391	3d	1.23				
"		2392	10d	.24				
"		2393	10d	.69				

INDUCTION- in vivo							
Mouse	Treatment	Tumor Wt	Vol Buffer	Reading	[TNF]pg/ml	[TNF]/tumor	[TNF]/n
2370	3d 899-6	0.45	1	0.679	331	480	1.0661
2371	3d 899-6	0.26	1	0.708	348	439	1.687
2372	3d 899-6	0.37	1	1.326	747	1023	2.7641
2373	3d 899-6	0.44	1	1.42	811	1168	2.6553
2374	10d 899-6	0.13	1	0.352	149	168	1.2938
2375	10d 899-6	0.54	1	0.444	197	304	0.5629
2376	10d 899-6	0.61	1	0.274	110	177	0.2897
2377	10d 899-6	0.23	1	0.323	134	165	0.717
2386	10d Control	2.41	1	2.021	1246	4249	1.7632
2387	10d Control	0.35	1	1.823	1099	1484	4.2401
2388	10d Control	0.47	1	2.498	1612	2370	5.0426
2390	3d 3616	0.44	1	0.37	158	228	0.5176
2391	3d 3616	1.23	1	0.515	236	527	0.4286
2392	10d 3616	0.24	1	0.374	160	199	0.8279
2393	10d 3616	0.69	1	0.756	377	637	0.9234

$$y = .005863 \times X$$

1. *Phragmites australis* (Cav.) Trin. ex Steud.

PLATE 17

DOI: 10.1002/for

G. D. LIMIT: 3 000

E. coli O157:H7 was isolated from ground beef samples collected from retail outlets in the United States.

READ MODE: OFFLINE SLOWDOWN

AUTO MEA

[illegible][illegible]

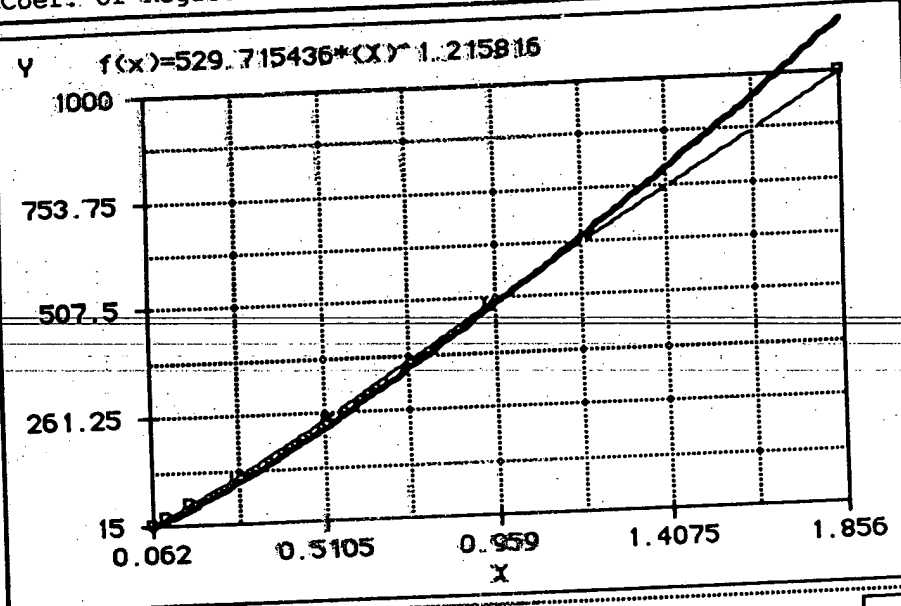
Modif./Inv. DATA:

.....X.....Y.....
0.062	15.13
0.095	31.13
0.158	62.5
0.282	125
0.518	250
0.943	500
1.856	1000

• EXPONENTIAL FIT •

$$Y = 529.715436 * (X)^{1.215816}$$

Coef. of Regression= 0.995013 For 7 Data Pairs



INITIALS Y.K. (5/26)

DATE 6/9/95

SUBJECT • Untitled : [I: X<>Y]



CPU Time: 13 Secs.



5/25/95

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1725							
1859	AA108972	RT Alone		13	13	11	
1862	AA108972	RT Alone		4.5	4	3	
1892	AA108964	RT Alone		4	4.5	2.5	
2189	AA113941	RT Alone		7	9.5	5.5	
2190	AA113941	RT Alone		9.5	10.5	7	
2191	AA113941	RT Alone					
2192	AA113941	RT Alone		9	10	6.5	
2201	AA113944	RT Alone		10	11	8.5	
2202	AA113944	RT Alone		5.5	4	2.5	
2203	AA113944	RT Alone		9	10	7.5	
2204	AA113944	RT Alone		11	11.5	7.5	
1863	AA108971	10^7 3616+RT		3.5	3	3	
1864	AA108971	10^7 3616+RT		13	12	7.5	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	0			
2074	AA108966	10^7 3616+RT		10	14	6	
2032	AA111326	10^7 3616+RT		6.5	7	5	
2033	AA111326	10^7 3616+RT		4	3	2	
2035	AA111326	10^7 3616+RT		7	8.5	5	
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		6	4.5	3.5	
2037	AA111327	10^7 3616+RT	<10 d52	0			
2039	AA111327	10^7 3616+RT		8	9	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	0			
1873	AA108969	10^7 899-6+RT		6	5	3.5	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	6	7.5	4	
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		8	6.5	5.5	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3	4.5	1.5	
2048	AA111330	10^7 899-6+RT	<10 d38	0			
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		13	9	9	

5/29/95 Control

2354 15.5 14.5 11.5 23.5
 55 20 15.5 12 21.2
 56 15.5 11 8.5 23.8
 57 13 16 10 21.0

3616+RT
 2032 8 6.5 7 23.9
 33 3 3.5 2 25.8
 2072 0 24.7
 35 9 8 6 23.8

EFFICIENCY LINE 22-206

	1	2	3	4	5	6	7	8	9	
1	RT					3616+RT				
2	2342	14	14	6	20.9	2036	5.5	4.5	4	25.1
3	43	12.5	10.5	8	22.3	37	0			26.5
4	44	10	10	8	20.6	2073	0			21.3
5	45	12	10	7	21.2	39	8	7	4	21.2
6	RT					3616+RT				
7	2350	14	11.5	8	21.7	1883	0			21.6
8	51	9.5	7	6	22.7	84	0			23.9
9	52	10	9	7	23.6	2074	15.5	10	7	16.9
10	53	14	9	8	20.6	86	0			21.3
11	RT					3616+RT				
12	2189	9	9.5	7	20.6	1863	4	4	3.5	24.2
13	90	7	6	4	18.4	64	13	11	7	22.6
14	92	9.5	9	5.5	24.1	65	0			26.3
15	2201	10	10	9	19.3	66	0			25.3
16	02	2.5	2.5	1	26.6	3616				
17	03	11.5	11	6	20.0	1723	0			
18	04	12	11	7.5	23.7	899-6				
19	RT					2054	0			23.2
20	1859	12	13	11.5	26.3	2075	0			24.2
21	1892	4.5	3	2.5	24.3	1713	0			22
22	1862	5	4.5	4.5	25.1	1710	0			24.4
23	RT					899+RT				
24	2346	13.5	10.5	10	19.2	1880	0			21.4
25	47	12	10	7.5	23.0	81	8	5	4	27
26	48	16	10	9	18.6	82	0			24
27	49	9	11.5	7	22	899 CRT				
28	CONTROL					2048	0			14.6
29	2206	17.5	20	16.5	24.9	50	0			25.3
30	07	13	14.5	11.5	24.9	51	14	10	9	25.1
31	CONTROL									
	2358	16	15.5	14	20.9					
	59	16	15.5	11.5	20.7					
	60	17.5	12	11	23.4					
	61	13	16	10	22					

RT Cont'd
 2354 17 17.5 11
 55 22 17.5 11.5
 56 16 14.5 8
 57 16.5 8.5 9

6/1/95

3616 + RT
 2032 8 8.5 6
 33 4 3 2.5
 2072 0
 35 9 7.5 6

EFFICIENCY LINE 22-206



	1	2	3	4	5	6	7	8	9
1	RT	2342	15.5	13.5	9.5	3616 + RT			
2		2343	12	9	7	1805	5	4	3
3		2344	13	1	7.5	01	12.5	10.5	7
4		2345	7.5	10	7.5	05	0		
5						06	0		
6	RT	2350	17	10	8.5	3616 + RT			
7		2351	11	8	7	1803	0		
8		2352	10	8	7	04	2	1	1
9		2353	14	9.5	7	? SAC → 2074	10	14	5.5
10	RT	2189	10	10	7	06	0		
11		2190	6	6	3.5	3616 + RT			
12		2192	9.5	10	5	2036	6	5	4
13	RT	2201	9	11	8	37	0		
14		2202	0			2073	0		
15		03	9.5	9.5	5	2039	8	7	4
16		04	11	11.5	7				
17	RT	1859	13	12.5	11.5	899-6 + RT			
18		1862	4	0	3	? SAC → 2048	0		
19		1863	5	5	4	2050	6	4	1
20						2051	12	15	9
21	RT	2346	14	12	8.5	899 + RT			
22		2347	12	11	8	1800	0		
23		2348	15	10	9	01	7	9.5	6
24		2349	11.5	10	7	02	0		
25	CONTROL					899-6 + RT			
26		2358	19.5	18	14	2054	0		
27		59	18.5	17.5	12	2075	0		
28		60	15	18	12	173	0		
29		61	15.5	17.5	12	170	0		
30	CONTROL					899-6 + RT			
31		2206	20	22	18	2044	8	8.5	7
		2207	14.5	14.5	13.5	45	ded		
						46	0		



The University of Chicago Departmental Purchase Order

Purchase Order Number
Z904417
THIS NUMBER MUST APPEAR ON ALL
PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS \$500.00.

Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

Vector Laboratories

30 Ingold Rd

Burlington, CA 94010

Payment Terms

State

Zip Code

Delivery charge? ☐ Yes ☐ No

Telephone No. 800-227-6666 FAX No. 415-697-0339

THE UNIVERSITY OF CHICAGO

Dept. Code:

Dept. Pathology

Coras Sibley, MD

5330 S. Ellis Ave

Chicago

State

Zip Code

Ship To

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under \$500.00, are used to exceed the restriction of \$500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.

2. Order MUST be typewritten.

3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.

4. Vendor and ship to must have complete addresses.

5. Department Code MUST be filled in with 4-digit department code from listing already furnished.

6. Only ONE account code is allowed per order.

Authorized Signature

Payroll No.

Order placed by phone? ☐ No ☒ Yes

Virginia
Order placed with (name)

6/2/85
Date

Print Name

Order placed by (name)

2-0294
Phone #

Account Code

Date

QTY

UNIT

DESCRIPTION

NET UNIT PRICE

ITEM TOTAL

1

Kit

PK-4002 Peroxidase restriction ABC - Mouse IgG

\$185.00

6.25

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS \$500.00

BACK ORDERS ARE NOT ALLOWED

ORDER TOTAL

\$191.25

6/5/95

J-013

Greg's Mouse Log							
Mouse #	Cage #	Group					weight
2207	AA113945	Control		18	16	11	26.4
2354	AA117027	Control ϕ		22	19	9	23.3
2356	AA117027	Control R		18	16.5	7	23.7
2357	AA117027	Control L		17	14	10	19.6
2359	AA117028	Control L		22	18	11	20.5
2360	AA117028	Control R	18	18	16	12	24.6
2361	AA117028	Control L		21	20	11	24.8
1710	AA105283	10 ⁺ 7 899-6	<10 d35	ϕ	ϕ	ϕ	24.7
2075	AA105283	10 ⁺ 7 899-6	<10 d42	ϕ	ϕ	ϕ	24.5
1713	AA105283	10 ⁺ 7 899-6	<10 d14	ϕ	ϕ	ϕ	21.5
2054	AA111331	10 ⁺ 7 899-6	<10 d49	ϕ	ϕ	ϕ	22.5
	105283						
1723	AA104549	10 ⁺ 7 3616	<10 d28 face	ϕ	ϕ	ϕ	26.5
1859	AA108972	RT Alone ϕ		15	14	10	24.8
1862	AA108972	RT Alone L		5	5	3	25.1
1892	AA108972	RT Alone R		4	4	2	24.1
2189	AA113941	RT Alone		10	10	4.5	24.0
2190	AA113941	RT Alone		5	5	2.5	20.9
2192	AA113941	RT Alone		9	9	3	25.2
2201	AA113944	RT Alone		8	8	3	19.6
2202	AA113944	RT Alone		ϕ	ϕ	ϕ	27.0
2203	AA113944	RT Alone		11	11	4.5	20.8
2204	AA113944	RT Alone		12	11.5	7	26.6
2342	AA117024	RT Alone		14	12	5	22.1
2343	AA117024	RT Alone		11.5	9	3.5	22.5
2344	AA117024	RT Alone		12.5	9	5	22.4
2345	AA117024	RT Alone		15	8.5	5	22.9
2346	AA117025	RT Alone		15	10	6	20.0
2347	AA117025	RT Alone		11.5	11.5	7	23.9
2348	AA117025	RT Alone		16	11	7	19.4
2349	AA117025	RT Alone		12	11	5	21.7
2350	AA117026	RT Alone ϕ		16	10	6	21.3
2351	AA117026	RT Alone L		10	8	4	23.4
2352	AA117026	RT Alone R		10	16	4	21.7
2353	AA117026	RT Alone L		13	9	1	22.1

Tues day: Δ Clog for biohazard animals Done ✓

1863	φ	AA108971	10^7 3616+RT		4	3	2	24.1
1864	L	AA108971	10^7 3616+RT		11	10	3	25.5
1865	R	AA108971	10^7 3616+RT	<10 d38	φ	φ	φ	26.4
1866	L	AA108971	10^7 3616+RT	<10 d31	φ	φ	φ	25.6
1883	φ	AA108966	10^7 3616+RT	<10 d24	φ	φ	φ	23.1
1884	L	AA108966	10^7 3616+RT	<10 d56	φ	φ	φ	23.4
1886	L	AA108966	10^7 3616+RT	<10 d31	φ	φ	φ	19.8
2074	R	AA108966	10^7 3616+RT		11	10	2	14.7
2032	φ	AA111326	10^7 3616+RT		9	9	5	25.0
2033	L	AA111326	10^7 3616+RT		4	3	2	26.2
2035	L	AA111326	10^7 3616+RT		10	7	3	25.1
2072	R	AA111326	10^7 3616+RT	<10 d21	φ	φ	φ	25.7
2036	φ	AA111327	10^7 3616+RT		7	5	3	24.8
2037	L	AA111327	10^7 3616+RT	<10 d52	2	2	2	27.2
2039	L	AA111327	10^7 3616+RT		8	8	3	22.1
2073	R	AA111327	10^7 3616+RT		φ	φ	φ	21.7
1871	φ	AA108969	10^7 899-6+RT	<10 d66	φ	φ	φ	24.8
1873	R	AA108969	10^7 899-6+RT		5.5	5	2	25.1
1874	L	AA108969	10^7 899-6+RT	<10 d52	φ	φ	φ	28.5
1880		AA108967	10^7 899-6+RT	<10 d14 L	φ	φ	φ	25.1
1881		AA108967	10^7 899-6+RT	<10 d42 R	8	9	3	27.5
1882		AA108967	10^7 899-6+RT	<10 d24 LR	φ	φ	φ	21.7
2044	φ	AA111329	10^7 899-6+RT		8	8	5	26.7
2046	R	AA111329	10^7 899-6+RT	<10 d77	φ	φ	φ	25.7
2048		AA111330	10^7 899-6+RT	<10 d38 φ	φ	φ	φ	13.5
2050		AA111330	10^7 899-6+RT	<10 d31 R	2	2	1	25.4
2051		AA111330	10^7 899-6+RT	LR	18	13	5	26.3

6/8/95
Greg's Mouse Log

Mouse #	Cage #	Group					weight
2207	AA113945	Control		15	16	14	
2354	AA117027	Control	SAC	23	22	13	
2356	AA117027	Control		19.5	17	11	
2357	AA117027	Control		18	15	12	
2359	AA117028	Control	SAC	23	20	15	
2360	AA117028	Control	SAC	18	17	14.5	
2361	AA117028	Control	SAC	22	23	16.5	
1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1723	AA104549	10^7 3616	<10 d28 face	0			
1859	AA108972	RT Alone	Sac'd ill	12	12	13	
1862	AA108972	RT Alone		4.5	6	4	
1892	AA108964	RT Alone		3.5	5	2.5	
2189	AA113941	RT Alone		-9.5	10	6.5	
2190	AA113941	RT Alone		5	4.5	2.5	
2192	AA113941	RT Alone		9.5	8	4	
2201	AA113944	RT Alone		8.5	7	3.5	
2202	AA113944	RT Alone		0			
2203	AA113944	RT Alone		10	9	5	
2204	AA113944	RT Alone		13	10.5	8.5	
2342	AA117024	RT Alone		12	10.5	6.5	5
2343	AA117024	RT Alone		12	10	7	
2344	AA117024	RT Alone		15	12.5	8	
2345	AA117024	RT Alone		-10.5	8.5	5.5	
2346	AA117025	RT Alone		13	11	9	
2347	AA117025	RT Alone		12	10.5	9	
2348	AA117025	RT Alone		14.5	10	8	
2349	AA117025	RT Alone		11	9.5	5	
2350	AA117026	RT Alone		-14.5	10.5	7	
2351	AA117026	RT Alone		10	8	7	
2352	AA117026	RT Alone		9.5	8	6.5	
2353	AA117026	RT Alone		13.5	8.5	7	

17
7
114
x18
952
1190

16
7
112
x15
160
1120

1863	AA108971	10^7 3616+RT		5	4	2.5		
1864	AA108971	10^7 3616+RT		11.5	9.5	6.5		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	0				
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT		14	10	6		
2032	AA111326	10^7 3616+RT		8	9.5	8		
2033	AA111326	10^7 3616+RT		4	3.5	2		
2035	AA111326	10^7 3616+RT		9	8	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		6	5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		7	6	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	0				
1873	AA108969	10^7 899-6+RT		6	5	4		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	8.5	10	6.5		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		8.5	7.5	6		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38 SAC	wt loss				
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT		21.5	16	10		

2209 4A113446 10^7 3616 8 6.5 7.5
 10 impl 5/3 inj 6/8 7.5 6.5 5.5
 11 10 7 6
 12 8 8.5 6

6/12/95

Greg's Mouse Log										
Mouse #	Cage #	Group								weight
2207	AA113945	Control			21	19	9			23.5
2354	AA117027	Control								
2356	AA117027	Control	dead							
2357	AA117027	Control	dead							
2359	AA117028	Control								
2360	AA117028	Control								
2361	AA117028	Control								
1710	AA105283	10^7 899-6	<10 d35		0					
2075	AA105283	10^7 899-6	<10 d42		0					
1713	AA105283	10^7 899-6	<10 d14		0					
2054	AA111331	10^7 899-6	<10 d49		0					
1723	AA104549	10^7 3616	<10 d28 face		0					
1859	AA108972	RT Alone								
1862	AA108972	RT Alone			5.5	6	7			25.1
1892	AA108964	RT Alone			5.5	3	2			25.3
2189	AA113941	RT Alone			9.5	11	7.5			24.8
2190	AA113941	RT Alone			3.5	3	2			20
2192	AA113941	RT Alone			8.5	8	4			24.2
2201	AA113944	RT Alone			6	7	4			22.3
2202	AA113944	RT Alone			0					27.8
2203	AA113944	RT Alone			9	10	4.5			26.5
2204	AA113944	RT Alone			14	11	8			25.5
2342	AA117024	RT Alone			14	15.5	6			23.6
2343	AA117024	RT Alone			10	9	6			23.4
2344	AA117024	RT Alone			12	10	6.5			24
2345	AA117024	RT Alone			10	12	4			23.8
2346	AA117025	RT Alone			13	9.5	7			19.4
2347	AA117025	RT Alone			12	11.5	8			23.4
2348	AA117025	RT Alone			15.5	9	8			18.4
2349	AA117025	RT Alone			13	10	7			20.7
2350	AA117026	RT Alone			14	9.5	7			21.8
2351	AA117026	RT Alone			12.5	8	6.5			22.9
2352	AA117026	RT Alone			9	8	5			23.4
2353	AA117026	RT Alone			10	8	5			24.3

1863	AA108971	10^7 3616+RT		5	4	3		23.8
1864	AA108971	10^7 3616+RT		9	8	4		26.5
1865	AA108971	10^7 3616+RT	<10 d38	0				25.8
1866	AA108971	10^7 3616+RT	<10 d31	0				25.8
1883	AA108966	10^7 3616+RT	<10 d24	0				22.8
1884	AA108966	10^7 3616+RT	<10 d56	1	1	1		21.5
1886	AA108966	10^7 3616+RT	<10 d31	0				19.6
2074	AA108966	10^7 3616+RT		19	10	7		15.8
2032	AA111326	10^7 3616+RT		8.5	8.5	7		24.3
2033	AA111326	10^7 3616+RT		3.5	3.5	2.5		26.6
2035	AA111326	10^7 3616+RT		8	9	4		24.9
2072	AA111326	10^7 3616+RT	<10 d21	0				25.9
2036	AA111327	10^7 3616+RT		4.5	5	3		22.8
2037	AA111327	10^7 3616+RT	<10 d52	0				25.9
2039	AA111327	10^7 3616+RT		7	4.5	2.5		21.2
2073	AA111327	10^7 3616+RT		0				21.1
1871	AA108969	10^7 899-6+RT	<10 d66	0				25.8
1873	AA108969	10^7 899-6+RT		4	7	3.5		26.1
1874	AA108969	10^7 899-6+RT	<10 d52	0				28.9
1880	AA108967	10^7 899-6+RT	<10 d14	0				20.4
1881	AA108967	10^7 899-6+RT	<10 d42	9	10.5	6		28.8
1882	AA108967	10^7 899-6+RT	<10 d24	0				24.3
2044	AA111329	10^7 899-6+RT		8	4.5	6		25.8
2046	AA111329	10^7 899-6+RT	<10 d77	0				25.4
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	4	2.5	1.5		26.2
2051	AA111330	10^7 899-6+RT	SOC	16	26	12		-

3616
 2209 10 8.5 6 25.2
 2210 9 7 5 25.2
 2211 13 9.5 9.5 21.8
 2212 8.5 9 6.5 22.6

6/15/95.

Greg's Mouse Log

Mouse #	Cage #	Group					weight
2207	AA113945	Control					
2354	AA117027	Control					
2356	AA117027	Control					
2357	AA117027	Control					
2359	AA117028	Control					
2360	AA117028	Control					
2361	AA117028	Control					
1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1723	AA104549	10^7 3616	<10 d28 face	0			
1859	AA108972	RT Alone					
1862	AA108972	RT Alone		5	6	4.5	
1892	AA108964	RT Alone		4.5	3	2.5	
2189	AA113941	RT Alone		10	13	6	
2190	AA113941	RT Alone		4	3	1.5	
2192	AA113941	RT Alone		8	7	4	
2201	AA113944	RT Alone		6	7	4	
2202	AA113944	RT Alone		0			
2203	AA113944	RT Alone		9	9	4	
2204	AA113944	RT Alone		13.5	11	9	
2342	AA117024	RT Alone		8	13.5	5.5	
2343	AA117024	RT Alone		10	9	6	
2344	AA117024	RT Alone		11.5	9	6.5	
2345	AA117024	RT Alone		13	14.5	9	
2346	AA117025	RT Alone		13	11	8	
2347	AA117025	RT Alone		10.5	10.5	7	
2348	AA117025	RT Alone		15	9.5	8.5	
2349	AA117025	RT Alone		10	14	7.5	
2350	AA117026	RT Alone		14	10.5	7	
2351	AA117026	RT Alone		10	8	6	
2352	AA117026	RT Alone		9.5	8.5	6	
2353	AA117026	RT Alone		12	9	6.7	

3016

2209

2210

2211

2212

1863	AA108971	10^7 3616+RT		4.5	3	2.5		
1864	AA108971	10^7 3616+RT		8.5	6	3		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	-1.5	1.5	1.5		
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT	sal wt loss	-2.4	12.5	7		
2032	AA111326	10^7 3616+RT		-8	8	6		
2033	AA111326	10^7 3616+RT		3	2	1.5		
2035	AA111326	10^7 3616+RT		8.5	8.5	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		5	4.5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		6	4.5	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	6	4.5	3		
1873	AA108969	10^7 899-6+RT		4.5	5.5	3.5		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	10	10.5	6		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		9	8	9		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT						

Greg's Mouse Log

Mouse #	Group				Weight		Weight
1710	10*7 899-6	0	-	-	26.7		
2075	10*7 899-6	0	0	0	26.2		
2213	10*7 899-6	0	-	-	23.0		
2054	10*7 899-6	0	-	-	25.1		
1725	10*7 3618	0	-	-	25.2		
1862	RT Alone	11	8	7	26.1		
1882	RT Alone	3	3	2	24.1		
2180	RT Alone	0	7	5	25.2		
2190	RT Alone	0	-	-	25.2		
2192	RT Alone	4	4	15	26.0		
2201	RT Alone	3	3	15	27.3		
2202	RT Alone	0	-	-	24.6		
2203	RT Alone	5	8	13	25.5		
2204	RT Alone	5	14	12	25.0		
2342	RT Alone	12	12	12	24.3		
2343	RT Alone	12	11	8	24.7		
2344	RT Alone	12	9	8	25.5		
2345	RT Alone	7	3	15	23.4		
2346	RT Alone	12	10	7	24.3		
2347	RT Alone	12	15	11	20.9		
2348	RT Alone	15	13	4	21.2		
2349	RT Alone	14	10	8	25.4		
2350	RT Alone	13	10	6	25.1		
2351	RT Alone	8	7	6	27.4		
2352	RT Alone	4	8	5	25.5		
2353	RT Alone	11	7	4	24.7		
1863	10*7 3618-RT	3	3	1	24.7		
1864	10*7 3618-RT	0	-	-	24.3		
1865	10*7 3618-RT	0	-	-	26.4		
1866	10*7 3618-RT	0	-	-	24.5		
1883	10*7 3618-RT	0	-	-	25.4		
1884	10*7 3618-RT	0	-	-	25.7		
1886	10*7 3618-RT	12	9	8	26.6		
2032	10*7 3618-RT	2	2	21	24.7		
2033	10*7 3618-RT	3	2	1	23.9		
2035	10*7 3618-RT	0	-	-	21.4		
2072	10*7 3618-RT	3	3	2	26.9		
2036	10*7 3618-RT	1	1	1	19.0		
2037	10*7 3618-RT	0	-	-	21.3		
2039	10*7 3618-RT	0	-	-	26.4		
2073	10*7 3618-RT	0	-	-	27.2		
1871	10*7 899-6-RT	0	-	-	25.6		
1873	10*7 899-6-RT	0	-	-	19.8		
1874	10*7 899-6-RT	0	-	-	27.2		
1880	10*7 899-6-RT	15	13	11	24.0		
1881	10*7 899-6-RT	8	-	-	26.4		
1882	10*7 899-6-RT	11	10	7	27.0		
2044	10*7 899-6-RT	0	-	-	27.0		
2046	10*7 899-6-RT	1	1	1	27.0		
2050	10*7 899-6-RT	1	1	1	27.0		

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Greg's Mouse Log									
Mouse #	Group							weight	weight
1710	10^7 899-6	0						25.8	
2075	10^7 899-6	0						25.2	
1713	10^7 899-6	0						22.7	
2054	10^7 899-6	0						24.3	
1723	10^7 3616	0						26.3	
1862	RT Alone	8	7	4				29.2	
1892	RT Alone	4	4	2				22.9	
2189	RT Alone	14	11	10				25.3	
2190	RT Alone	0						22.0	
2192	RT Alone	6	6	6				25.0	
2201	RT Alone	5	5	4				28.0	
2202	RT Alone	0						27.4	
2203	RT Alone	9	9	6				23.3	
2204	RT Alone	18	17	10				27.6	
2342	RT Alone	13	12	6				25.0	
2343	RT Alone	12	7	7				26.3	
2344	RT Alone	19	15	9				25.3	
2345	RT Alone	10	8	5				25.3	
2346	RT Alone	11	11	8				22.8	
2347	RT Alone	15	14	9				26.0	
2348	RT Alone	16	9	7				18.3	
2349	RT Alone	16	16.5	6				22.2	
2350	RT Alone	15	11	10				25.5	
2351	RT Alone	9	8	5				24.2	
2352	RT Alone	9	9	4				24.7	
2353	RT Alone	11	12	6				27.3	
1863	10^7 3616+RT	4	4	2				25.3	
1864	10^7 3616+RT	4	3	1.5				25.6	
1865	10^7 3616+RT	0						27.4	
1866	10^7 3616+RT	0						26.7	
1883	10^7 3616+RT	0						23.4	
1884	10^7 3616+RT	0						19.5	
1886	10^7 3616+RT	0						22.6	
2032	10^7 3616+RT	0						26.0	
2033	10^7 3616+RT	4	3	1.5				26.5	
2035	10^7 3616+RT	7	6	4				24.7	
2072	10^7 3616+RT	9	7	5				26.8	
2036	10^7 3616+RT	6	5	1.5				23.9	
2037	10^7 3616+RT	0						27.3	
2039	10^7 3616+RT	5	5	1.5				19.9	
2073	10^7 3616+RT	0						22.5	
1871	10^7 899-6+RT	0						25.9	
1873	10^7 899-6+RT	7	6	1.5				26.1	
1874	10^7 899-6+RT	0						27.0	
1880	10^7 899-6+RT	0						19.8	
1881	10^7 899-6+RT	15	12	7				32.1	
1882	10^7 899-6+RT	0						23.9	
2044	10^7 899-6+RT	14	14	7				28.2	
2046	10^7 899-6+RT	0						26.3	
2050	10^7 899-6+RT	0						26.4	

Data Sheet

Mouse#	Group	Measurements	Weight
2140	RT Alone	6x6x3	7x6x3
2190	RT Alone	0x	0
2192	RT Alone	2x2x1	1x1x1
2201	RT Alone	2x2x1.5	3x3x1.5
2202	RT Alone	0x	0
2203	RT Alone	4x4x3	4x3x3
2204	RT Alone		
2342	RT Alone	11x10x3	11x9x3
2343	RT Alone	12x10x2	11x10x2
2344	RT Alone	13x12x1.5	11x11x1.5
2345	RT Alone	7x11x1.5	6x13x1.5
2346	RT Alone	12x10x2	9x7x5
2347	RT Alone	13x12x1.5	17x15x11
2348	RT Alone	13x12x1.5	16x14x8
2349	RT Alone	14x10x10	14x10x8
2350	RT Alone	12x9x8	12x9x6
2351	RT Alone	8x6x5	9x7x4
2352	RT Alone	9x8x4	8x9x3
2353	RT Alone	11x6x5	9x6x4
2687	RT Alone	12x10x8	11x9x7
2688	RT Alone	13x11x9	14x10x8
2689	RT Alone	13x8x8	14x8x7
2690	RT Alone	10x7x5	9x7x6
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
2667	Control	19x16x15	
2668	Control	16x15x12	16x14x13
2669	Control	16x15x11	18x16x13
2670	Control	22x15x12	22x15x10

26.7

23.4

25.9

29.2

29.9

23.5

24.3

24.4

24.0

24.5

23.4

26.3

21.9

17.4

26.1

21.9

26.0

25.1

21.0

23.9

24.5

11x10x7

12x11x8 21.8

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18.5

23.0

Data Sheet

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7/17/95

2679	10^7	3636RT	6x6x3	5x5x2
2680	10^7	3616RT	8x6x3	7x4x2
2681	10^7	3636RT	12x10x7	11x9x4
2682	10^7	3616RT	13x8x8	13x9x6
2691	10^7	3636RT	13x10x9	11x8x8
2692	10^7	3616RT	11x8x8	11x10x8
2693	10^7	3696RT	13x10x8	11x10x6
2694	10^7	3616RT	12x9x9	11x9x6
2695	10^7	3616RT	8x6x6	8x7x4
2696	10^7	3636RT	7x7x5	6x5x3
2697	10^7	3616RT	8x7x5	6x4x3
2698	10^7	3616RT	10x7x4	8x6x6
2675	10^7	8996RT	10x9x7	8x8x8
2676	10^7	8996RT	12x12x9	13x9x8
2677	10^7	8996RT	14x9x8	12x4x4
2678	10^7	8996RT	14x9x9	13x10x6
2671	10^7	8996RT	8x6x4	6x5x4
2672	10^7	8996RT	9x7x6	8x6x5
2673	10^7	8996RT	10x10x9	13x11x6
2674	10^7	8996RT	10x8x9	9x8x1.5
2695	10^7	8996RT		10x8x8
2696	10^7	8996RT		17x12x9
2697	10^7	8996RT		10x8x7
2698	10^7	8996RT		11x11x6
2663	899-6		13x10x9	16x12x8
2664	899-6		25x13x15	12x25x15
2665	899-6		10x11x6	
2666	899-6		14x9x8	19x15x10
2703	899-6		9x7x5	11x7x4
2704	899-6		11x9x6	11x11x3
2705	899-6		14x12x10	18x14x7
2706	899-6		16x11x9	17x11x9
	899-6			
	899-6			
	899-6			
	899-6			

24.5
23.2
22.6
26.7
26.4
17.1
25.1
19.3

10x7x5

SHC 7/17

11x7x4

Data Sheet

2/13/95 2/17/95

2659	3616	14x11x4	15x15x8
2660	3616	22x16x13	28x19x11
2661	3616	19x15x11	18x16x7
2662	3616	23x15x15	22x17x12
2701	3616	13x10x7	
2708	3616	12x9x6	
2709	3616	11x9x7	
2710	3616	13x11x8	
2715	3616	9x8x6	
2716	3616	12x8x7	
2721	3616	9x8x7	
2722	3616	10x8x6	

AA12000

AA12000

5 AC 1/1

5 AC 1/1

10/20/95

2/18/95

Data Sheet

Greg's Mouse Data			
Mouse#	Group	Measurements	Weight
2189	RT Alone	9 x 5 x 1.5	25.8
2190	RT Alone	0	23.9
2192	RT Alone	3 x 3 x 2	25.5
2201	RT Alone	5 x 2 x 1.5	25.1
2202	RT Alone	0	24.3
2203	RT Alone	4 x 3 x 1.5	24.2
2204	SAC	RT Alone	
2342	RT Alone	11 x 4 x 4	24.4
2343	RT Alone	11 x 2 x 6	24.4
2344	RT Alone	22 x 16 x 10	24.0
2345	RT Alone	6 x 3 x 1.5	24.3
2346	RT Alone	7 x 8 x 5	23.5
2347	RT Alone	16 x 16 x 10	25.2
2348	RT Alone	15 x 15 x 10	21.0
2349	RT Alone	died 7/10/15	
2350	RT Alone	8 x 8 x 3	24.3
2351	RT Alone	12 x 9 x 5	27.1
2352	RT Alone	8 x 8 x 3	25.7
2353	RT Alone	9 x 6 x 4	25.3
2687	RT Alone	12 x 9 x 6	22.0
2688	RT Alone	12 x 10 x 6	23.5
2689	RT Alone	12 x 8 x 5	24.7
2690	RT Alone	9 x 7 x 6	25.1
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
2667	SAC	Control	
2668	Control		
2669	Control	19 x 14 x 14	22.7
2670	Control	21 x 13 x 8	17.4

died 7/20/15

Data Sheet

		2/20/95	2/20/95
		12/1	12/1
2679	10^7 3636RT	5 x 4 x 1.5	12.4
2680	10^7 3616RT	7 x 4 x 1.5	13.2
2681	10^7 3636RT	11 x 7 x 5	24.2
2682	10^7 3616RT	13 x 8 x 8	20.0
2691	10^7 3636RT	7 x 6 x 4	23.5
2692	10^7 3616RT	9 x 8 x 6	24.1
2693	10^7 3636RT	12 x 10 x 3	22.8
2694	10^7 3616RT	13 x 9 x 7	24.4
2683	10^7 3616RT	7 x 6 x 4	23.2
2684	10^7 3636RT	0	19.0
2685	10^7 3616RT	6 x 3 x 1.5	22.4
2686	10^7 3616RT	10 x 8 x 4	14.9
2675	10^7 8996RT	12 x 8 x 5	25.2
2676	10^7 8996RT	13 x 12 x 7	17.3
2677	10^7 8996RT	12 x 9 x 5	14.1
2678	10^7 8996RT	12 x 10 x 6	25.8
2671	10^7 8996RT	7 x 7 x 4	22.3
2672	10^7 8996RT	8 x 6 x 4	25.1
2673	10^7 8996RT	14 x 11 x 6	20.1
2674	10^7 8996RT	11 x 8 x 4	14.4
2695	10^7 8996RT	10 x 9 x 6	22.7
2696	10^7 8996RT	17 x 13 x 9	19.2
2697	10^7 8996RT	10 x 10 x 6	24.9
2698	10^7 8996RT	12 x 10 x 8	17.1
2663	899-6	16 x 15 x 7	22.4
2664	SAC 899-6		
2665	899-6	21 x 17 x 13	24.3
SAC 2666	899-6		
2703	899-6	13 x 8 x 5	25.2
2704	899-6	12 x 11 x 4	27.3
2705	899-6	21 x 15 x 9	26.3
2706	899-6	19 x 13 x 6	21.0
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	899-6		

LA V 10 0-251

10/1/95

real

0 PFU (100)

0.1 PFU (100)

10.10 PFU (100)

10.10 PFU (100)

real 9 plates plus 1 plate for control

10 up 12 plates

EFFICIENCY 0.22/205

$$\frac{1.9}{2.8} \times \frac{100}{100}$$

1) Prep

2) Count cells $148 \times 2 \times 10^4 = 16.8 \times 10^5 = 16 \times 10^6$ cells per 100 ml

3) Add 100 ml cells/plate + 1/2 ml cell toxin

10/10/95

Plates 80-90% confluent

Count cells in 100 ml plate 73000

$$80 \times 12 \times 10^4 = 960 \times 10^4 = 9.6 \times 10^6 = 10 \times 10^6$$

will infect 0.1 PFU and 1 PFU

$$\frac{0.1 \text{ PFU } (10 \times 10^5)}{\text{PFU}} \times 10 \times 10^5 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^5 \text{ PFU}} \right) = \frac{5}{100} \times \frac{10000}{1 \text{ ml}} = 50 \text{ ml}$$

100 ml in 9 ml

$$\frac{1 \text{ PFU } (10 \times 10^5)}{\text{PFU}} \times 10 \times 10^5 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^5 \text{ PFU}} \right) = \frac{1.1}{100} \times \frac{10000}{1 \text{ ml}} = 11 \text{ ml/plate}$$

4.4 ml in 12 ml

infected 94% cm

10/11/95

2) Harvest 4 plates 94% cm

3) Harvest 2 plates 1.2 4.5 7.8 2.6

4) Plates 3, 4, 9 2.4 2.8 2.0 2.0 1.1 ml/plate

1st visit 1-82 (yabbies cool)

15/1/95

2nd visit 2-01-1995 at 11:30 am
2° post 1st add 2nd round media

1st visit

16/1/95

1st visit at 3:00 am (-20°)

2nd visit at 2:00 pm (6° post 21) and 3:00 am 16/1/95

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1st visit at 2:00 3:30 am

16/1/95

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D-54 (66N) in radio wire cylinder

10/11/95

decide 3 wire 1000 MVA to DSH in (1) wall

	early	(1) wire	(1) wire
SD08	0	11x10x9	11x10x9
SD09	1	12x11x8	12x10x12
SD10	12	12x10x8	12x9x8

in 1000 MVA 1000 (3x10x10) per hour

In radio wire

10/11/95

In radio 3 wire 20 G to (1) wall
 & SD07 partially inside 2 to minor size

10/12/95

Decide

	L_{max}	L	$L(a)$	L
Day 1 SD08	11x10x9	11x10x9	5.13	5.15
			5.56	5.62
			0.43	.47g
Day 2 SD09	12x11x8	12x10x12	5.17	5.11
			6.73	7.06
			1.56	1.98
SD10	12x10x8	12x9x6	5.11	5.03
			5.78	5.89
			0.70	.36

10/11/64

10/11/64

Report of repl. on 10/11/64 (the repl. has
data will be given
1/5 in 6 per infection
0.1 PFU

0.1 PFU

0.1 PFU

1.0 PFU

Plate ~ 2×10^6 cells / 100mm plate
in 10ml media

10/12/65

cells ~ 80% confluent

$2.0 \times 10^9 = 7 \times 10^6$ cells / plate

1 PFU 7×10^6 PFU 1ml at 1)

9×10^9 PFU

4 x 10¹² (2%)
3ml / plate

0.1 PFU

1ml 1 PFU + 9ml media (2%)

10⁶
after 20, then add 2ml 10% media
LT of post infection ~ 4.5

Harvest at 10¹⁵ 1.2 4.5 7.8
10% 20% 20%

4.5 3 6 10/11/65
1 50% 20%

Data Sheet

7/20/95

2659	3616	20 x 20 x 14	24 8
2660 SAC	3616		
2661	3616	21 x 16 x 12	23 2
2662 SAC	3616		
2707	3616		
2708	3616		
2709	3616		
2710	3616		
2719	3616		
2720	3616		
2721	3616		
2722	3616		

Data Sheet

Greg's Mouse	Data	7/21/95	7/27/95	
Mouse #	Group	Measurements	Weight	
2189	RT Alone	8x8x1.5	5x5x1.5	25.3
2190	RT Alone	Ø	Ø	25.9
2192	RT Alone	Ø	Ø	23.3
2201	RT Alone	5x3x1.5	5x3x1.5	29.8
2202	RT Alone	Ø	Ø	29.1
2203	RT Alone	5x4x1.5	6x4x1.5	23.3
2204	SAC	RT Alone		
2342	RT Alone	10x17x12	22x17x12	24.6
2343	RT Alone	12x10x5	12x10x6	24.7
2344	RT Alone	Ø	Ø	23.1
2345	RT Alone	Ø	Ø	24.7
2346	RT Alone	11x7x3	8x7x3	25.1
2347	RT Alone	15x15x10	17x15x10	26.0
2348	RT Alone	15x15x9	14x11x9	20.8
SAC 2349	RT Alone			
2350	RT Alone	12x9x6	10x9x5	27.2
2351	RT Alone	10x8x4	10x7x3	25.5
2352	RT Alone	9x9x1.5	9x9x1.5	26.2
2353	RT Alone	10x7x2	9x8x4	25.7
2687	RT Alone	11x9x6	11x10x4	28.6
2688	RT Alone	12x9x5	12x9x4	25.3
2689	RT Alone	11x9x8	12x9x4	24.3
2690	RT Alone	10x8x4	9x7x4	27.3
2354	Control			
2355	Control			
2356	Control			
2357	Control			
2358	Control			
2359	Control			
2360	Control			
2361	Control			
2667	SAC	Control		
2668	Control			
2669	Control			
2670	Control			

Data Sheet

7/14/95		7/27/95	
2650	3616	21x21x15	
2660 GAO	3616		
2661	3616	25x11x13	
2662 SAC	3616		
2707	3616	11x9x7	11x9x7 23.8
2708	3616	12x10x6	12x10x5 22.8
2709	3616	14x12x7	14x13x10 25.0
2710	3616	13x11x7	14x11x8 25.0
2719	3616	7x7x4	6x5x15 22.0
2720	3616	11x9x5	8x6x3 25.1
2721	3616	7x6x4	13x5x6 23.3
2722	3616	9x8x4	9x6x4 22.7

Data Sheet

Greg's Mouse		Data		
Mouse #	Group	Measurements	Weight	
2189	RT Alone	Ø	Ø	24.5
2190	RT Alone	Ø	Ø	22.4
2192	RT Alone	Ø	Ø	25.0
2201	RT Alone	Ø	Ø	28.0
2202	RT Alone	5x3x1.5	Ø	28.4
2203	RT Alone	4x4x1.5	4x4x1.5	22.9
SAC 2204		RT Alone		
2342	RT Alone	23x19x7	23x11x9	21.0
2343	RT Alone	11x10x6	12x11x5	23.5
2344	RT Alone	Ø	Ø	23.0
2345	RT Alone	Ø	Ø	24.5
2346	RT Alone	Ø	Ø	24.4
2347	RT Alone	19x17x11	14x17x11	21.3
2348	RT Alone	14x12x10	14x12x10	26.8
SAC 2349		RT Alone		21.6
2350	RT Alone	9x8x3	7x8x3	24.4
2351	RT Alone	8x7x3	9x6x2	25.4
2352	RT Alone	10x9x3	8x8x1.5	26.6
2353	RT Alone	9x5x1.5	8x5x2	25.5
2687	RT Alone	10x7x4	11x7x5	24.0
2688	RT Alone	9x9x4	11x11x4	25.6
2689	RT Alone	14x13x6	15x13x9	24.1
2690	RT Alone	8x7x2	7x7x3	26.9
2679	10^7 3636RT	Ø	Ø	22.7
2680	10^7 3616RT	Ø	Ø	
2681	10^7 3636RT	Ø 9x7x3	6x6x2	15.0
2682	10^7 3616RT	12x16x4	12x6x5	21.5
2691	10^7 3636RT	4x4x1.5	3x3x1.5	23.2
2692	10^7 3616RT	10x6x3	8x6x2	27.7
2693	10^7 3636RT	8x7x3	8x7x1.5	23.4
2694	10^7 3616RT	11x9x4	11x9x4	25.9
2683	10^7 3616RT	6x4x1.5	3x3x1.5	23.3
2684	10^7 3636RT	Ø	Ø	24.8
2685	10^7 3616RT	Ø	Ø	25.1
2686	10^7 3616RT	5x5x2	4x4x1.5	20.8
2675	10^7 8996RT	7x5x1.5	6x5x2	27.1
2676	10^7 8996RT	10x9x4.5	8x8x4	19.1
2677	10^7 8996RT	7x6x2	5x4x1.5	22.0
2678	10^7 8996RT	8x8x3	8x8x3	28.0

Data Sheet

2/17/95

2671	10*7	8996RT	2x2x1.5	2x2x1.5	22.2
2672	10*7	8996RT	5x3x1.5	2x2x1.5	25.5
2673	10*7	8996RT	9x7x3	9x7x2	18.6
2674	10*7	8996RT	6x4x1.5	4x3x1.5	22.9
2695	10*7	8996RT	3x6x3	6x5x2	21.8
2696	10*7	8996RT	4x4x2	10x5x1.5	20.9
2697	10*7	8996RT	9x7x6	7x7x3	22.5
2698	10*7	8996RT	10x9x5	11x9x4	16.8
2663	899-6		16x11x5	17x13x6	24.2
SAC 2664	899-6				
SAC 2665	899-6				
SAC 2666	899-6				
2703	899-6		21x16x7	21x19x7	24.3
2704	899-6		22x16x6	23x15x5	14.5
2705	899-6		18x15x8		
2706	899-6		18x18x6	17x15x4	19.7
2711	899-6		15x13x8		
2712	899-6		13x10x8		
2713	899-6		11x11x10		
2714	899-6		14x10x10		
2707	3616		13x10x4	15x13x4	27.2
2708	3616		11x10x6	12x11x6	23.4
2709	3616		17x15x7	20x16x9	26.9
2710	3616		17x13x7	17x15x10	25.7
2719	3616		5x3x1.5	2x2x1.5	23.9
2720	3616		5x4x1.5	2x2x1.5	26.3
2721	3616		11x9x3	13x11x6	24.8
2722	3616		7x6x2	6x6x4	24.5

die 8/1/95

injected
8/1/95

Data Sheet

8/7/95

8/10/95

Greg's Mouse		Data	
Mouse#	Group	Measurements	Weight
2189	RT Alone	1.5 x 1.5 x 1	6.0 x 1.0 x 1
2190	RT Alone	Ø	Ø
2192	RT Alone	Ø	Ø
2201	RT Alone	Ø	Ø
2202	RT Alone	Ø	Ø
2203	RT Alone	1.5 x 1.5	1.5 x 1.5 x 1.5
SAC 2204 RT Alone			
2342	RT Alone	22 x 19 x 9	20 x 15 x 8
2343	RT Alone	12 x 10 x 4	12 x 12 x 6
2344	RT Alone	Ø	Ø
2345	RT Alone	Ø	Ø
2346	RT Alone	Ø	Ø
2347	RT Alone	20 x 18 x 9	20 x 18 x 9
2348	RT Alone	15 x 11 x 6	15 x 12 x 6
SAC 2349 RT Alone			
2350	RT Alone	8 x 7 x 3	8 x 6 x 4
2351	RT Alone	10 x 7 x 3	9 x 7 x 4
2352	RT Alone	10 x 9 x 2	8 x 8 x 2
2353	RT Alone	8 x 5 x 2	8 x 5 x 3
2687	RT Alone	11 x 8 x 5	9 x 8 x 5
2688	RT Alone	10 x 9 x 4	10 x 9 x 5
2689	RT Alone	12 x 12 x 9	15 x 12 x 12
2690	RT Alone	9 x 6 x 3	7 x 6 x 3
2679	10^7 3616RT	Ø	Ø
SAC 2680 10^7 3616RT			
2681	10^7 3636RT	12 x 8 x 5	12 x 9 x 5
8/4/95 2682 10^7 3616RT			
2691	10^7 3636RT	7 x 5 x 1.5	Ø
2692	10^7 3616RT	Ø	6 x 6 x 1.5
2693	10^7 3636RT	7 x 7 x 1.5	8 x 6 x 1.5
2694	10^7 3616RT	10 x 7 x 4	10 x 7 x 3
2683	10^7 3616RT	Ø	Ø
2684	10^7 3636RT	Ø	Ø
2685	10^7 3616RT	Ø	Ø
2686	10^7 3616RT	4 x 3 x 1.5	1.0 x 1.0 x 1
2675	10^7 8996RT	4 x 4 x 1.5	4 x 4 x 1.5
2676	10^7 8996RT	8 x 8 x 4	9 x 7 x 4
2677	10^7 8996RT	1.5 x 1.5 x 1.5	4 x 4 x 1.5
2678	10^7 8996RT	9 x 8 x 3	8 x 6 x 3

HISTO-PATHOLOGICAL

3d 6d 9d
 Control
 RT alone
 RT + 3616
 3616 alone

12

Data Sheet

Greg's Mouse		Data	
Mouse#	Group	Measurements	Weight
2342	RT Alone	20x14x8	20x14x8
2343	RT Alone	14x10x7	12x11x7
2344	RT Alone	Ø	Ø
2345	RT Alone	Ø	Ø
2346	RT Alone	Ø	Ø
SAC 2347	RT Alone	20x14x8	20x14x8
2348	RT Alone	14x14x6	10x14x6
SAC 2349	RT Alone	20x14x8	20x14x8
2350	RT Alone	10x8x4	10x8x4
2351	RT Alone	9x6x2	8x7x3
2352	RT Alone	11x7x2	9x7x3
2353	RT Alone	7x6x2	7x5x2
2687	RT Alone	9x7x4	10x7x4
2688	RT Alone	10x7x4	10x10x4
2689	RT Alone	13x11x3	13x12x5
2690	RT Alone	8x6x3	8x6x3
2679	10^7 3636RT	Ø	Ø
SAC 2680	10^7 3616RT	20x14x8	20x14x8
2681	10^7 3636RT	12x10x6	12x10x9
SAC 2682	10^7 3616RT	20x14x8	20x14x8
2691	10^7 3636RT	Ø	Ø
2692	10^7 3616RT	6x6x1.5	6x5x1.5
2693	10^7 3636RT	6x5x1.5	5x4x1.5
2694	10^7 3616RT	8x6x2	8x6x2
2683	10^7 3616RT	Ø	Ø
2684	10^7 3636RT	Ø	Ø
2685	10^7 3616RT	Ø	Ø
2686	10^7 3616RT	1.0x1.0x1.0	Ø
2675	10^7 8996RT	4x4x1.5	4x4x1.5
2676	10^7 8996RT	10x8x4	8x9x6
2677	10^7 8996RT	4x3x1.5	6x4x1.5
2678	10^7 8996RT	8x6x3	7x6x2
2671	10^7 8996RT	Ø	Ø
2672	10^7 8996RT	Ø	Ø
2673	10^7 8996RT	5x5x1.5	4x4x1.5
2674	10^7 8996RT	Ø	Ø
2695	10^7 8996RT	3x3x1.0	2x2x1.5
2696	10^7 8996RT	11x10x6	11x10x4
2697	10^7 8996RT	7x5x2	10x4x2

Data Sheet

Greg's Mouse		Data		
Mouse#	Group	Measurements	Weight	
2346	RT Alone	ϕ	ϕ	25.3 ϕ
SAC 2347	RT Alone	-	-	
2348	RT Alone	16x14x7	17x14x8	21.7
SAC 2349	RT Alone	-	-	
2350	RT Alone	9x7x3	8x6x3	22.3
2351	RT Alone	8x6x3	8x7x3	25.8
2352	RT Alone	7x7x2	10x8x3	28.1
2353	RT Alone	7x5x3	7x5x2	26.3
2357	RT Alone	9x8x3	10x8x3	25.8
2358	RT Alone	8x11x10x4	11x9x4	28.9
2359	RT Alone	15x12x4	16x13x5	27.8
2360	RT Alone	8x6x3	7x5x3	28.2
2679	10^7 3636RT	ϕ	ϕ	25.3
SAC 2680	10^7 3616RT	-	-	
2681	10^7 3636RT	13x12x9	13x12x8	21.0
SAC 2682	10^7 3616RT	-	-	
2691	10^7 3636RT	6x5x1.5	6x4x1.5	29.2
2692	10^7 3616RT	ϕ	ϕ	22.1
2693	10^7 3636RT	4x4x1.5	4x3x1.5	24.7
2694	10^7 3616RT	6x5x2	6x6x2	27.0
2683	10^7 3616RT	ϕ	ϕ	24.0
2684	10^7 3636RT	ϕ	ϕ	23.3
2685	10^7 3616RT	ϕ	ϕ	26.2
2686	10^7 3616RT	1x1	ϕ	22.1
2675	10^7 8996RT	6x6x2	5x5x2	27.5
2676	10^7 8996RT	9x9x4	10x9x5	21.4
2677	10^7 8996RT	5x3x1.5	5x3x1.5	24.2
2678	10^7 8996RT	6x5x2	6x5x1.5	29.1
2671	10^7 8996RT	ϕ	ϕ	23.8
2672	10^7 8996RT	ϕ	ϕ	27.8
2673	10^7 8996RT	4x2x1.5	4x1.5x1.5	26.2
2674	10^7 8996RT	ϕ	ϕ	24.5
2695	10^7 8996RT	1x1x1	1x1x1	28.5
2696	10^7 8996RT	11x10x6	10x9x5	23.0
2697	10^7 8996RT	7x5x2	8x5x3	25.3
2698	10^7 8996RT	11x8x5	12x10x4	17.1
dead	2714 899-6	13x16x13	-	

8/22/95

Data Sheet

2719	3616	Φ	Φ	23.9
2720	3616	6x3x1.5	8x4x2	27.2
SAC 2721	3616	4x4x1.6		
2722	3616	15x15x6	15x15x6	24.9
2715	HSV/GAN	18x15x11	20x18x10	deal 8/23
2716	HSV/GAN	21x18x10	23x18x15	21x17x11 21.8
2717	HSV/GAN	14x11x8	14x13x9	25.9
2718	HSV/GAN	22x18x10	23x18x15	28.6
2731	HSV/RT/GAN	6x6x1.5	6x6x3	23.4
2733	HSV/RT/GAN	13x13x4	12x11x3	23.6

Data Sheet

8/28/15

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2346	RT Alone	Ø	
SAC 2347	RT Alone	20x14x7	
2348	RT Alone	16x14x7	
SAC 2349	RT Alone	20x14x7	
2350	RT Alone	15x6x3	
2351	RT Alone	1x7x3	
2352	RT Alone	8x3x5	
2353	RT Alone	9x4x3	
2687	RT Alone	11x8x4	
2688	RT Alone	9x6x4	
2689	RT Alone	10x15x8	
2690	RT Alone	6x6x2	
2679	10^7 3636RT	Ø	
SAC 2680	10^7 3616RT	13x11x7	
2681	10^7 3636RT	13x11x7	
SAC 2682	10^7 3616RT	13x11x7	
2691	10^7 3636RT	Ø	
2692	10^7 3616RT	4x3x1.5	
2693	10^7 3636RT	1x1x0.5	
2694	10^7 3616RT	3x3x1.5	
2683	10^7 3616RT	Ø	
2684	10^7 3636RT	Ø	
2685	10^7 3616RT	Ø	
2686	10^7 3616RT	Ø	
2675	10^7 8996RT	4x3x2	
2676	10^7 8996RT	2x8x3	
2677	10^7 8996RT	4x3x1.5	
2678	10^7 8996RT	1x1x0.5	
2671	10^7 8996RT	Ø	
2672	10^7 8996RT	Ø	
2673	10^7 8996RT	1x1x0.5	
2674	10^7 8996RT	Ø	
2695	10^7 8996RT	Ø	
2696	10^7 8996RT	9x8x3	
2697	10^7 8996RT	6x6x3	
2698	10^7 8996RT	12x10x5	
SAC	2714 899-6	20x14x7	

8/24/15

Data Sheet

8/25/95

2719	3616	0	
2720	3616	6x6x3	
SAC 2721	3616	10x15x5	
2722	3616	10x15x5	
5/24/95 SAC 2723	HSV/GAN		
2718	HSV/GAN	18x16x8	
2717	HSV/GAN	18x13x7	
5/24/95 SAC 2718	HSV/GAN		
2735	HSV/GAN	14x12x6	
2731	HSV/RT/GAN	5x4x3	
2733	HSV/RT/GAN	10x10x3	

① 2732 injected 5/18/95 9x4x6

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2687	RT Alone	11x9x3	25.5
2688	RT Alone	8x8x3	29.3
2689	RT Alone	14x17x8	27.8
2690	RT Alone	8x6x3	28.5
2679	10^7 3636RT	Ø	15.6
SAC 2680	10^7 3616RT	13x13x7	20.7
2681	10^7 3636RT	13x13x7	20.7
SAC 2682	10^7 3616RT	13x13x7	20.7
2691	10^7 3636RT	Ø	23.5
2692	10^7 3616RT	Ø	30.2
2693	10^7 3636RT	Ø	15.1
2694	10^7 3616RT	9x9x2	25.8
2683	10^7 3616RT	Ø	25.5
2684	10^7 3636RT	Ø	23.7
2685	10^7 3616RT	Ø	26.7
2686	10^7 3616RT	Ø	21.8
2675	10^7 8996RT	5x5x3	21.5
2676	10^7 8996RT	8x6x3	23.7
2677	10^7 8996RT	5x3x1.5	21.4
2678	10^7 8996RT	8x8x0.5	30.4
2671	10^7 8996RT	Ø	24.8
2672	10^7 8996RT	Ø	21.2
2673	10^7 8996RT	1x1x0.5	22.1
2674	10^7 8996RT	Ø	25.6
2695	10^7 8996RT	1x1x1	24.7
2696	10^7 8996RT	8x8x3	23.7
2697	10^7 8996RT	11x9x5	26.2
2698	10^7 8996RT	13x11x6	20.0
2719	3616	Ø	24.3
2720	3616	7x7x3	29.4
SAC 2721	3616	13x13x7	20.7
2722	3616	19x15x6	26.6
SAC 2715	HSV/ GAN	20x16x8	21.9
2716	HSV/ GAN	20x16x10	21.9
2717	HSV/ GAN	24x18x10	26.7
SAC 2718	HSV/ GAN	20x16x8	21.9

8/21/95

9/4/95

9x7x3
7x6x3
20x18x6
3x5x2

13x13x8

9x7x2

4x4x3

2x6x4

4x2x1.5

1x1x0.5

4x4x1

1x6x2

13x9x6

13x13x5

Ø

10x9x6

20x13x8

20x16x8

24x20x13

Data Sheet

9/2/95 9/10/95

Mouse#	Group	Measurements	Weight	
2687	RT Alone	9x6x3	26.1	7x5x2
2688	RT Alone	7x5x2	28.9	10x6x4
2689	RT Alone	14x16x4	24.5	13x14x6
2690	RT Alone	8x6x4	29.7	6x5x2
2679	10^7 3636RT	12x10	15.8	0
SAC 2680	10^7 3616RT	10x10x10	20.0	mm
2681	10^7 3636RT	15x13x8	21.7	15x14x8
SAC 2682	10^7 3616RT	10x10x10	20.0	mm
2691	10^7 3636RT	0	24.2	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.0	0
2694	10^7 3616RT	7x7x2	25.0	10x8x4
2683	10^7 3616RT	0	25.8	0
2684	10^7 3636RT	0	23.8	0
2685	10^7 3616RT	0	27.2	0
2686	10^7 3616RT	0	22.2	0
2675	10^7 8996RT	5x4x3	29.7	1x3x2
2676	10^7 8996RT	6x6x4	24.8	5x5x2.5
2677	10^7 8996RT	0.5x0.5x0.5	25.7	0.5x0.5x0.5
2678	10^7 8996RT	1x1x0.5	30.5	0.5x0.5x0.5
2671	10^7 8996RT	0	24.8	0
2672	10^7 8996RT	0	28.3	0
2673	10^7 8996RT	0	27.6	0
2674	10^7 8996RT	0	25.5	0
2695	10^7 8996RT	7x5x2	26.2	8x6x3
2696	10^7 8996RT	9x6x3	23.4	5x3x1.5
2697	10^7 8996RT	13x11x6	27.0	15x10x7
2698	10^7 8996RT	15x15x5	20.0	15x15x6
2719	3616	0	26.3	0
2720	3616	10x10x2	27.3	11x10x6
SAC 2721	3616	10x10x10	20.0	mm
SAC 2722	3616	19x21x10	—	mm
SAC 2715	HSV/ GAN	18x12x8	29.7	16x11x6
2716	HSV/ GAN	21x18x8	22.9	23x18x10
SAC 2717	HSV/ GAN	18x12x8	29.7	16x11x6
2718	HSV/ GAN	18x12x8	29.7	16x11x6

Data Sheet

9/2/95

9/10/95

2730	HSV/GAN		
2731	HSV/RT/GAN	3x2 x 1.5	25.6
2733	HSV/RT/GAN	5x5 x 1.5	25.8
2732	HSV/RT/GAN	13x10 x 8	22.0

3x2 x 1.5
0
12x8x6

Data Sheet

Greg's Mouse		Data			
Mouse #	Group	Measurements	Weight		
2687	RT Alone	11.5 x 2	26.4	6 x 5 x 1.5	
2688	RT Alone	18 x 17 x 7	24.6	8 x 10 x 8	
2689	RT Alone	6 x 6 x 4	30.2	5 x 5 x 4	
2690	RT Alone	6 x 4 x 2	28.1	6 x 4 x 2	
2679	10^7 3616RT	0	26.3	0	
SAC 2680	10^7 3616RT	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
2681	10^7 3616RT	6 x 11 x 6	21.7	11 x 13 x 7	
SAC 2682	10^7 3616RT	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
2691	10^7 3616RT	0	25.1	0	
2692	10^7 3616RT	0	30.8	0	
2693	10^7 3616RT	0	26.8	0	
2694	10^7 3616RT	10 x 9 x 6	21.7	12 x 10 x 6	
2683	10^7 3616RT	0	26.1	0	
2684	10^7 3616RT	0	24.0	0	
2685	10^7 3616RT	0	27.6	0	
2686	10^7 3616RT	0	22.4	0	
2675	10^7 8996RT	5 x 4 x 3	29.3	5 x 5 x 1	
2676	10^7 8996RT	0	25.4	0	
2677	10^7 8996RT	0.5 x 0.5 x 0.5	25.3	0.5 x 0.5 x 0.5	
2678	10^7 8996RT	0.5 x 0.5 x 0.5	30.1	0	
2671	10^7 8996RT	0	25.2	0	
2672	10^7 8996RT	0	28.8	0	
2673	10^7 8996RT	0	28.0	0	
2674	10^7 8996RT	0	26.0	0	
2695	10^7 8996RT	1 x 6 x 3	25.2	1 x 5 x 2	
2696	10^7 8996RT	0	23.0	0	
2697	10^7 8996RT	14 x 10 x 6	21.3	14 x 10 x 6	
2698	10^7 8996RT	15 x 13 x 7	19	16 x 14 x 4	
2719	3616	0	25.1	0	
2720	3616	13 x 12 x 5	28.4	14 x 11 x 5	
SAC 2721	3616	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
SAC 2722	3616	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
2715	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
2716	HSV/ GAN	12 x 10 x 5	26.7	12 x 6 x 4	
2717	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	
2718	HSV/ GAN	XXXXXXXXXXXXXXXXXXXX	XXXXXXXXXXXX	XXXXXXXXXXXX	

Data Sheet

9/14/85

SAC	2735	HSV/GAN	24 x 21 x 14	14.3
	2731	HSV/RT/GAN	0.5 x 0.5 x 0.5	26
	2733	HSV/RT/GAN	0	23.7
	2732	HSV/RT/GAN	11 x 9 x 6	23.2

9/18/85

0

0

10 x 9 x 5

Sibley @ ndmc.duke.edu

10

[illegible]

EFFICIENCY LINE 22.203



11/1/85

U-37 Cell Culture

Wash Start U-37 culture - frozen cell line (1/1/85)
for injection into mice

Media: penicillin streptomycin - Call's (1/29/85)

500 cc DMEM

8/21/85

50 cc pen (100x)

5 cc penicillin

5 cc streptomycin

5 cc HEPES

Methods:

Place frozen cell aliquot into warm H₂O bath ^{concent}
Transfer cells to 5 ml of media in 10 ml flask drop by drop
Spin in centrifuge for 5' at 300-500 rpm
Aspirate off supernatant & section
Resuspend cells in 2 ml of media (drop by drop)
Add 2 ml of cells to 3 ml in T-25 (drop by drop)
Place cells in incubator (top slightly loosened)

9/20/85

C-6 (SODM) in milk

Harvest milk from M.J. of C-6 (P) in milk

car by

① humer

② humer

S601
S602
S603
S604

g milk 1.40 g
① LR
g milk 1.40 g
② LR

inject milk c 10ml 3616 g per liter

Radiation of C-6 node

only 2 mice could be radiated due to humer size
radiated for 20 Gy (10' 28")
irradiated ① and ② LR

Harvest C-6 node milk humer in 2 mice (day 1)

	wt ①	wt ②
will harvest	S601	S602
	11x9x6 mm	12x7x7
	19x15x11 mm	19x15x11 mm
	Blank	Blank
	5.11 g	5.16 g
	5.33 g	5.33 g
	5.17 g	5.17 g
	5.17 g	5.14 g
	7.12 g	7.12 g
	2.02 g	1.98 g

Day 3 (9/26/85)

day 3 ← S603
due to humer size

21x16x6

23x20x16

5.15 5.21
6.6 7.6
7.4 8.3
5.0 5.0
5.0 5.0
5.0 5.0

Day 7 (9/28/85)

S6004

22x16x15

17x14x14

5.0 5.0
5.0 5.0
5.0 5.0

D-54 (684) sk. male mice 4/10/55

Harvest mice from M.D. of D-54 in (A) hind limb

early

(1) tumor (mm³)

(2) tumor (mm³)

5005

1

3.0 x 2.6 = 0

14 x 11 (36)

5006

1.6

11 x 7 x 6 (46)

12 x 6 x 4 (30)

5007

2

11 x 9 x 7 (69)

14 x 8 x 7 (78)

inject mice a total 3616 (2440 + 1176) per above

10/4/55

Irradiation of D-54 mice

irradiate 3 mice a total to (12) hind limb

Harvest of D-54 mice tumor

10/5/55

Harvest 5007 on day 1 (10/5/55), 5005-5006 on day 2 (10/10/55)

5007

(1) mm³

14 x 9 x 8

(2) mm³

16 x 9 x 9

wt (g)
(3)

liver

5.16

(4)

5.03

liver tumor

5.93

5.51

tumor

0.77

0.48

5005

Belmont

11 x 6 x 5

liver

5.8

5.22

liver tumor

6.36

5.54

tumor

1.12

0.32

5006

22 x 15 x 12

16 x 12 x 9

liver

5.18

5.05

liver tumor

6.08

6.31

tumor

1.80

1.26

1. V. 10 D-59 (Gibco)

10/8/95

need

0 PFU

(0.5)

(10)

(1)

1 PFU

(0.5)

(10)

(1)

10 PFU

(0.5)

(10)

(1)

need 9 plates plus 1 plate
set up 12 plates

1) Tappin's Cells

2) Count Cells

$$127 \times 12 \times 10^4 = 144 \times 10^5 = 1.44 \times 10^6$$

resuspend in 4 ml media

3) Add 1 ml media/plate and 1 ml cells soln

Infect plates 4-9 w/ H3N2 (1 PFU or 10 PFU)

10/8/95

~90% confluent

1) Count cells in 1 plate

$$30 \times 10^4 \times 10^4 = 3 \times 10^9$$

for 1 PFU want 3×10^6 PFU/plate

for 10 PFU " 3×10^7 PFU/plate

$$[3616] = 9 \times 10^9 \text{ PFU/ml}$$

$$\frac{3 \times 10^6 \text{ PFU/plate}}{9 \times 10^9 \text{ PFU/ml}} = \frac{1 \text{ ml}}{3 \times 10^3} \left(\frac{1000 \text{ ml}}{1 \text{ ml}} \right)$$

$$= \frac{1}{3} \text{ ml/plate}$$

plates 4, 5, 6
3 PFU, not 10 PFU
need 3 plates
set up 9

add virus
1 ml in 20 ml
media

$$\text{plates 7, 8, 9} \quad \frac{3 \times 10^7 \text{ PFU/plate}}{9 \times 10^9 \text{ PFU/ml}} = \frac{1 \text{ ml}}{3 \times 10^2} \left(\frac{100 \text{ ml}}{1 \text{ ml}} \right)$$

$$= \frac{1}{3} \times 10 \text{ ml/plate}$$

$$= 3.33 \text{ ml/plate virus}$$

Add Virus 11:30 10/8/95 in 3 ml 20 media

set up 4

$$= 13.33 \text{ ml in 12 media}$$

Dr. Vito G. M. Gylfina

10/6/95

need

0 PFU

0.5g

2g (3.4g)

0.1 PFU

0.5g

2g (3.4g)

10 PFU

0.5g

2g (3.4g)

infect 12 plates plus 1 for count cells
 = 0 plates = by to sel 12 plates

1) Trypsinize cells

2) Count cells

$$60 \times 24 \times 10^4 = 14.4 \times 10^6$$

in 10ml

3) Try to plate 2×10^6 cells / plate

4) Add 0.5ml cell soln to 9ml media / plate

0.1 PFU, 1 PFU

10/9/95

Infect plates 4-9 with HSV-1 (1 PFU or 10 PFU)

~80% confluent

1) Count cells in 1 spot plate

47-49 ~ 50

$$50 \times 10 \times 10^4 = 5 \times 10^6 \text{ cells / plate}$$

infect 20 PFU (5×10^6 PFU) + 10 PFU (5×10^7 PFU)

2×10^7 PFU/ml

7.3.9

100 9×10^7 cells

1 PFU

$$5 \times 10^6 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^7 \text{ PFU}} \right) = \frac{2.5}{10} \text{ ml} = 0.25 \text{ ml}$$

2.5 ml = 250 μ l / plate

2ml in 12ml

in 3ml media

750 \times in 12ml

$$10 \text{ PFU } 5 \times 10^7 \text{ PFU} \left(\frac{1 \text{ ml}}{9 \times 10^9} \right) = \frac{1}{2} \times \frac{1}{100} \text{ ml} \times \frac{1000 \mu\text{l}}{1 \text{ ml}} = 5 \mu\text{l}$$

in 3ml

make 12ml add 20ml virus

4.5.6

0.1 PFU

$$5 \times 10^5 \text{ PFU} \left(\frac{1 \text{ ml}}{2 \times 10^9 \text{ PFU}} \right) = \frac{2.5}{100} \text{ ml}$$

2.5 ml

25 μ l / plate

75 \times in 9ml